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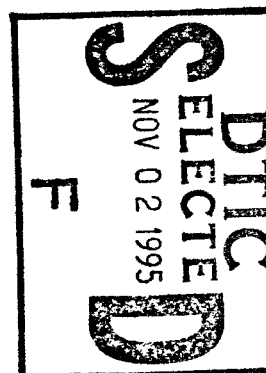
CHROMATOGRAPHY OF
POLYMERS
AND RELATED SUBSTANCES

ABSTRACTS OF PAPERS

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Main Lectures

Special Lectures

POSTERS

RECENT ADVANCES AND FUTURE DIRECTIONS IN SIZE EXCLUSION CHROMATOGRAPHY DETECTION SYSTEMS

H. G. Barth, Ch. Jackson and R. Lew

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As we enter the twenty-first century, polymeric materials are becoming more complex, frequently consisting of polymer blends, composites, and branched and grafted structures of unusual architecture. As a result, we are faced with unprecedented analytical challenges in which MW and average chemical composition may no longer provide sufficient information for process and quality control or for fundamental studies involving structure-property relationships. To meet these challenges, size exclusion chromatographic systems are being developed and utilized that consist of multiple detectors attached to the end of the SEC column. A modern system may consist of an online viscometer, light scattering detector, concentration-sensitive detector, and possibly a spectrophotometer. With such a configuration, it is feasible to determine accurately and precisely many structural features of a polymer, in addition to its MWD. Not only can one obtain average values, but also distributive properties as a function of MW, such as branching, chain conformation, and chemical composition.

In this talk, we will present an overview of new developments regarding concentration, composition, and molecular-weight sensitive detectors. Emphasis will be placed on the use and limitations of evaporative light scattering detection, FTIR systems, and combined viscometry and light scattering detectors. Other, less commonly used, online detectors will also be discussed.

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IS THE UNIVERSAL CALIBRATION STILL VALID?

H. Benoit, FRANCE

EFFECT OF ELECTROSTATIC AND HYDROPHOBIC INTERACTIONS ON THE CHROMATOGRAPHIC BEHAVIOR OF BIOPOLYMERS IN HPLC COLUMNS

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Biopolymers are complex molecules bearing hydrophobic, polar and charged functionality which are involved to different extents in the organization and maintenance of their three-dimensional structure through the formation of inter- and intramolecular hydrophobic and electrostatic bonding. The same hydrophobic and electrostatic forces maintaining the three-dimensional structure of biopolymers are also involved in the chromatographic retention of these biomolecules in the different modes of interactive high performance liquid chromatography (HPLC). The different modes of interactive HPLC are traditionally identified by the predominant type of interaction taking place between the surface area of the biopolymer exposed to the eluent and the surface of the functional groups bound to the chromatographic support that determine the chromatographic retention. In practice, however, more than one interaction can be involved in the mechanism of retention leading to mixed mode retention behavior that cause difficulty in the clear interpretation of retention data but may significantly enhance the selectivity of the chromatographic system. In hydrophobic interaction chromatography (HIC), for instance, using stationary phases having ionic groups at the surface, electrostatic interactions may affect the retention of proteins despite the relatively high salt concentration in the mobile phase. In size exclusion chromatography (SEC), biopolymers are theoretically separated by size and shape, with large excluded molecules eluting first and small, totally included molecules eluting last. In practice, depending on pH and composition of the mobile phase, silica based bonded stationary phases may exhibit weakly anionic or hydrophobic character leading to chromatographic behavior not related to the molecular size. It is important, therefore, to identify the mode of interaction and either attenuate or take advantage of mixed mode retention mechanisms when useful. In both cases the appropriate selection of the composition of the mobile phase is needed. Furthermore, alterations in the three-dimensional structure of biopolymers occurring during the chromatographic process may also affect their retention behavior lowering both peak efficiency and recovery of the biological activity of bioactive molecules.

This communication describes the effect of varying the composition of the mobile phases used in various modes of biopolymer liquid chromatography on the magnitude of hydrophobic and electrostatic interactions that take place either between the solute and the stationary phase or between the solute and the components of the mobile phase. The presentation includes the description of methods developed to characterize the chromatographic behavior of different HPLC columns and to tailor their selective properties for a given separation problem. It is shown that the selectivity of an HPLC column can be varied by selecting a predominant retention mechanism through the suppression or the enhancement of either the hydrophobic or the electrostatic character of the stationary phase, obtained by varying the composition of the mobile phase, changing the pH or the ionic strength or using a suitable additive.

LIQUID CHROMATOGRAPHY TECHNIQUES FOR CHARACTERIZING POLY(VINYL ALCOHOL)

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For copolymers having molar mass and composition distributions, characterization with one or more concentration detectors on-line to a chromatographic system based on size exclusion chromatography (SEC) produces average composition data. When copolymers contain composition heterogeneities, a cross-fractionation procedure, involving separating by composition by a method based on liquid chromatography (LC) fractions which were previously separated according to size, can be attempted. In coupled column chromatography (CCC), on-line transfer between SEC and LC techniques can be automated.

Poly(vinyl alcohol) (PVOH) represents an important class of water-soluble polymers. PVOH is produced by hydrolysis of poly(vinyl acetate) (PVAC) and has many applications dependent on the extent of hydrolysis between 50 and 100 per cent. Properties are also dependent on the method of hydrolysis, since partially hydrolysed samples resulting from alkaline hydrolysis have a blocky chain architecture, whereas acid hydrolysis yields a more random distribution of VAC units. Therefore, characterisation of partially hydrolysed PVOH may be considered to be a copolymer problem, for which distributions in molar mass, composition, sequence length and branching may occur. Our work to date has concentrated on SEC and LC separations with the aim in due course of developing CCC.

Aqueous SEC separations of PVOH samples having levels of hydrolysis above 70 per cent have been performed with columns containing PLaquagel-OH. Association and interaction effects involving VAC units in aqueous media required careful choice of eluent in order to minimise non size exclusion behaviour. Aqueous SEC experiments have been carried out using several eluents including standard electrolytes and surfactants. The most favourable molecular size separation was obtained using 0.25 per cent (w/v) sodium lauryl sulfate (SLS) as eluent. From chromatograms reasonable results for molar masses of partially hydrolysed PVOH were obtained with polysaccharide standards as SEC calibrants.

Compositional LC separations for partially hydrolysed PVOH were
established with columns containing the reversed phase packing PLRP-S
4000Å and an evaporative light scattering detector. Gradient elution
with water/tetrahyrofuran produced separations according to VOH/VAC
composition. The peaks observed for partially hydrolysed PVOH were
relatively broad indicating distributions of composition. Elution
behaviours of alkaline and acid hydrolysed samples exhibited a
dependence on sequence length distribution (blockiness). LC
separations with non-porous beads of PLRP-S demonstrated that
separations proceeded without interference from exclusion effects.

Initial CCC experiments showed the feasibility of on-line transfer for
automating the determination of size and composition distributions.
Further work in progress is directed towards optimising the CCC
system.

CANCELLED

CHROMATOGRAPHY OF COLLOIDAL SYSTEMS

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When we think of suspensions of latex, gold particles or even of micelles, it is evident how different the nature of colloids can be. Their characterisation is important for polymer chemists as well as for inorganic and physical chemists. Chromatographic methods are often favoured because they offer several advantages: In most cases the result is a size distribution, no average size; the method is fast, not very expensive and can be automated.

The chromatographic analyses of organic colloids have a rather long tradition. However, since the renaissance of metallic and semiconductor nanoparticles with their extraordinary properties, when their sizes became smaller and smaller down to 1.3nm and solar cells were constructed from such nanostructures, there is a need for the characterization of inorganic colloids. The present paper is focused more on this group, because these applications might be less well known, but the other group is also regarded for comparison.

The size exclusion chromatography (SEC) seems to be ideal for the determination of the size distribution, as the method is based on the ability to access into the pores of the stationary phase and is therefore directly related to the size. By SEC not only the hydrodynamic volume of latexes and micelles as well as the aggregation number of the latter can be determined [1,2], but also the same technique works effectively for inorganic colloids such as SiO_2[3] CdS [4,5], ZnS [6] and Au [7] in aqueous and organic solutions [8] . Short analysis times (4min) are crucial for rapidly growing particles. However, there are limitations and problems. When *HPLC* -SEC with 7µm material or smaller is utilized, inorganic particles bigger than about 20nm are filtered off. With some kinds of colloids, adsorption causes severe problems due to the charged surface of the particles. Therefore stationary and mobile phases must be carefully selected. In the liquid close to the solid/liquid interface of colloidal particles an electrical double layer of oppositely charged ions as compared to the surface charge is formed, as long as the liquid contains electrolytes. This double layer acts as a protection against coagulation and adsorption. It is quite rigid and has to be added to the size of the solid particle, when the effective particle size in SEC is considered. This phenomenon also strongly affects the recovery [9]. Moreover, the thickness of this layer varies with the ionic strength and can be determined by SEC, because the solid particle is rigid and does not shrink or swell as charged polymers do. On the other hand ionic strength is a crucial parameter in SEC, since the retention time is strongly affected by it. Chromatography also allows to study the interesting size depending properties of the inorganic nanoparticles e.g. optical absorption and fluorescence by using appropriate detectors. Preparative application is possible, though here the situation is much more complicated than in any other chromatographic method [10].

The classical hydrodynamic chromatography (HDC) utilises the laminar flow in capillaries of 25µm i.d. and below. With its centre of gravity a small species can reach zones closer to the wall than a big one. As a result species elute in the order of decreasing size giving also evidence for the size distribution. Latexes [11] and micelles [12] have been investigated by this method. In practice it might be difficult to analyse particles in the low nm-range [13]. If no commercial equipment is available, much experience is necessary for this technique and sensitivity problems may occur. In order to overcome these problems HDC is also carried out in columns packed with non-porous spheres [14].

Wide bore HDC operating in capillaries with internal diameters of several hundred µm is a very simple method for fast determination of the average particle size. The mechanism is based on the differently effective exchange of small and big particles between central zones in a laminar flow and those close to the wall due to the different diffusion coefficients. The advantages are: cheap equipment (peristaltic pump is sufficient) and much less problems with adsorption because of reduced surface. The *wide bore* HDC has been applied for polymer solutions, latexes [15] and inorganic colloids [16]. Both HDC techniques are not suitable for preparative separations.

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MN-L-6

FIELD FLOW FRACTIONATION IN INVESTIGATION OF COMPLEX POLYMER SYSTEMS

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MASS DETECTION IN GEL PERMEATION CHROMATOGRAPHY
USING A SINGLE CAPILLARY VISCOMETER

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COMPOSITIONAL CHARACTERIZATION OF STYRENE COPOLYMERS BY NON-EXCLUSION LIQUID CHROMATOGRAPHY

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INTRODUCTION
    Copolymers have both molecular weight and chemical composition distributions. Although a size exclusion chromatography(SEC)/dual detector system is well known as one of the methods of determining the latter distribution, only the average composition can be detected by SEC with two detectors [1]. Separation techniques of copolymers according to composition by high-performance liquid chromatography(HPLC) have been reported within the decade and SEC-HPLC is considered to be a preferable procedure for the determination of both distributions at a time. HPLC techniques will be reviewed here at first and then, the technique developed in our laboratory will be explained.

OUTLINE OF OUR METHOD
Preliminary experiment.
    Liquid adsorption chromatography(LAC) developed in our laboratory consists of silica gel/a mixture of chloroform (or 1,2-dichloroethane(DCE)) and ethanol[2]. Silica gel with a pore diameter of 3 nm and a mean particle size of 5 µm was packed in 4.6-mm i.d. x 50-mm-length stainless-steel tubing. This column was thermostated at a specified temperature. Gradient elution was performed at a flow rate of 0.5 mL/min. A UV detector was used at a wavelength of 260 nm (or 254 nm) and/or 233 (in the case of DCE) [3]. Samples tested in our method were styrene copolymers of methacrylates, acrylates, vinyl acetate, and acrylonitrile, in addition to ethyl methacrylate-butyl methacrylate copolymers.
    First, elution was performed by an isocratic elution mode. At a constant column temperature, the copolymers were retained in the column with chloroform(and DCE) without ethanol. By the addition of ethanol to chloroform (and DCE), copolymers having less MMA started to elute, and with increasing ethanol content in the mobile phase, those having more MMA were eluted. The copolymers tend to be adsorbed on the column at higher column temperature, and those having more MMA required a lower column temperature for elution. Lower column temperature (and/or much ethaol content in the mobile phase) was preferable for the elution of the copolymers having much MMA.

Gradient elution
    In order to change the retention volume of the copolymers of different composition, gradient elution mode was required at a specified column temperature [4]. Under the gradient elution condition that mobile phase A was chloroform / ethanol (99/1), B was chloroform/ethanol (95.5/4.5), and 100% A to 100% B in 15 min, the copolymers having MMA from 25% to 60% could be separated in the order of increasing MMA content in the copolymers at column temperature of

80 °C and those having MMA from 40% to 90% at column tempe-
rature of 30 °C. P(S-MMA) of narrow chemical composition
distributions still had the compositional difference of
several percents [4]. Fractionation by SEC followed by LAC
gave both molecular weight and chemical composition distri-
butions. P(S-MMA) having a broad chemical composition dis-
tribution was separated by SEC and each fraction was, then,
separated by LAC [5].
      Styrene copolymers of methyl, ethyl, and n-butyl acry-
lates and methacrylates were also separated according to
their composition by LAC[6]. Plots of the relationships for
copolymers of styrene-acrylate and styrene-methacrylate
having the same ester group lay roughly on the same line,
indicating that a pair of copolymers having the same ester
groupand the same styrene content could notbeseparated[7].
      Other styrene copolymers which are feasible for hydro-
gen bonding to the silica surface such as styrene-methyl
methacrylate block copolymers [8] and styrene-vinyl acetate
block copolymers [9] were also separated by LAC. Styrene-
acrylonitrile copolymers can also be separated composition.

Detection of methacrylate (and acrylate) component.
      DCE was transparent at wavelengths over 230 nm and
methacrylate (and acrylate) homopolymers and copolymers
could be monitored with a UV detector around 233 nm [3].
Ethyl methacrylate and n-butyl methacrylate homopolymers and
copolymers were separated at column temperature of 60 °C by
gradient elution from a mixture of DCE/ethanol(99/1) to
DCE/ethanol(90/10) in 20 min[10].
      The molar absorption coefficients for both PS and PMMA
at 233 nm were nearly equal and chromatograms obtained at
this wavelength reflected the relative amounts of the copo-
lymers with different chemical compositions, and direct
characterization of a chemical composition distribution from
the chromatograms was possible with minor modification [11].
      The use of two UV detectors or a UV-diode array detec-
tor monitored at 233 and 260 nm makes the direct determina-
tion of a chemical composition distribution possible. The
ratio of UV absorption intensities at 260 nm and 233 nm
represents the styrene content in copolymers such as P(S-
MMA). This method is more precise than the alternative
procedure which uses a calibration curve of styrene content
vs. retention volume constructed using the copolymers of
known compositions, because the peak broadening problem
which is commonly encountered in SEC influences the precise
estimation of a chemical composition distribution.

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THE ROLE OF CHROMATOGRAPHY IN STRUCTURAL BIOLOGY
Pore Morphology and Assembly

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Porous morphologies occur in distinctly different complexities: (semi-)rigid matrices and gels (fixed pores), concentrated solutions (fluctuating pores), dynamic structures maintained by energy consumption (rearrangement of porous structure coupled to metabolism), compartmentalization (three-dimensional bicontinuous morphologies separated by porous membranes).

Materials used in chromatography are of the simplest category and therefore may serve as model systems for other, compounded physical phenomena. Size exclusion Chromatography (SEC) even serves as porosimetry technique, because the mechanism of SEC is symmetrical in respect to the properties of the porous material and of the solutes immersed. One may characterize morphology via known solutes (called inverse SEC) or characterize unknown solutes based on a known retention-calibration graph. In practice the latter is still done via calibration with known solutes but once we understand the physical interelationships better we may have absolute criteria based on matrix morphology. One focus of this review therefore is on inverse SEC and Universal Calibration.

The limitations of structural biology, which mainly relies on imaging techniques, are well recognized. Because of widespread structual polymorphism specimen preparation may have changed morphology and therefore other techniques have to confirm the validity of morphological conclusions. These solution structure techniques have inherently lower resolution (in respect to information content) but are suitable to physiological and other conditions that cannot be studied by imaging techniques directly. One technique suited to study gross morphology of biological material is diffusion, since frictional drag depends on the relation between solute and pore size. The present review will discuss how chromatographic investigations in fact helped to establish this functional relationship which is crucial in absolute pore size determination via diffusion.

Structural biology also deals with isolated components and here a key question for solution structure techniques is size and shape, which is readily answered by SEC. The last part of this review therefore provides examples of otherwise conventional applications of chromatography based on Universal Calibration.

QUANTITATION IN THE ANALYSIS OF POLYMERS AND OLIGOMERS WITH DIFFERENT DETECTORS

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The goals of any chromatographic analysis are a good separation of the components of a sample, their identification, and their accurate quantitative determinatioh. While this may be comparatively easy with low molecular compounds, a quantitatively accurate characterization of polymers is complicated by the fact, that a sufficient resolution of molecules with different degree of polymerization (and - in the case of copolymers - chemical composition) can only be achieved for lower oligomers. Moreover, detector sensitivity typically varies with molar mass and chemical composition, and preferential solvation of the polymer coils can influence the accuracy of the results. The suitability and performance of typical LC detectors in polymer characterization depends on the nature of the samples. Depending on the type of detector, different corrections have to be made in order to obtain accurate results. In general, corrections of response factors for their molar mass dependence have to be made not only for the lowest oligomers. In the characterization of copolymers, multiple detection (using combinations of different concentration detectors) is generally inevitable, as it yields additional information on chemical composition and thus allows an accurate quantitation. Recently, MALDI-TOF-MS has become a powerful tool in polymer characterization. Despite its great advantages (speed, high resolution, identification of polymer molecules) this promising technique suffers, however, from considerable limitations with respect to quantitation.

LIGHT SCATTERING AND CHROMATOGRAPHY: RECENT DEVELOPMENTS

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The first combination of an on-line laser light scattering detector by Ouano and Kaye in 1974 using a Chromatix detector ushered in a new era for macromolecular characterization. Despite the intrinsic appeal of this concept, since it permits the absolute (i.e. no calibration standards) determination of molecular weights and their distributions, a great number of improvements both in chromatography and detection were required to allow its implementation with relative ease. These past few years have brought about significant advances in the application of this technique. Recent developments are reported following a brief review of the fundamentals of the light scattering measurement. Important developments reviewed include the detection and quantitation of branching, precise measurement of the polydispersity of so-called "standards", conformation plots, precise extraction of M-H-S coefficients by combining LS and viscosity, reverse phase to detect and quantify multimeric formation, flow FFF for an alternative separation combined with LS, TREF (temperature rising elution fractionation) measurements, capillary electrophoresis combined with LS, and the quantitation of aggregation phenomena and microgel formation.

CHARACTERIZING POLYMERS USING HPLC - FTIR TECHNIQUES

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Infrared spectroscopy combined with HPLC/SEC (size exclusion chromatography)has found wide acceptance as a tool in the study of deformulation of samples of unknown origin, compositional distribution in copolymers, impurity profiling in polymers, and branching in polyolefins. Use of this technique is enhanced by the availability of new commercial instrumentation and by software that speeds data analysis. The new instruments are off-line solvent removal devices that provide samples in a simple, solvent free condition that are compatible with existing FTIR instrumentation. There are two solvent removal approaches; the first uses a pneumatic nozzle with heated gas evaporation system, and the second uses an ultrasonic/vacuum nebulizer. Both remove much of the labor of preparing samples and both work with the normal sampling methods and concentration levels expected in SEC chromatography.

In addition to more powerful hardware, software is available for the analysis of time resolved spectra produced by the combined technique. This software, called 3D/IR, consists of a series of program modules and screen presentations reducing the large blocks of data into useful chemical and structural information about polymer samples. The process of reducing data to information then becomes a quick and easy task.

This presentation will include a discussion of the available commercial instrumentation, representative examples of studies from a variety of polymers, and examples of the qualitative and quantitative uses of 3D/IR software.

LIQUID CHROMATOGRAPHY OF HIGH POLYMERS UNDER CRITICAL CONDITIONS OF ADSORPTION: QUERIES CONCERNING EXPERIMENTAL FEASIBILITY OF METHOD

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Liquid chromatography (LC) of macromolecules under critical conditions of adsorption (LCCC) is based on the assumption that a balance may exist between (entropic) exclusion or permeation effects and (enthalpic) adsorption effects for macromolecules transported along appropriate column in an appropriate eluent. As known, the exclusion effects lead to the separation according to the size of macromolecules and the largest species elute first. In contrast, macromolecules of a given chemical nature elute in the reversed order when subject to adsorption on a LC column packing, i.e. the smallest species elute first. When a full balance between exclusion and adsorption is reached - "the critical point of adsorption" - the entropic and enthalpic effects mutually eliminate and macromolecules are no more separated according to their size. In order words, polymer molecules eluted at the critical point of adsorption leave the column at the same retention volume which does not depend on their molar mass and which equals to the total volume of liquid within column. One can say that macromolecules are "chromatographically invisible".

Above situation gives rise to interesting separation possibilities: If a polymer sample is constituted of two chemically different parts, like blocks in a block copolymer, components in a polymer mixture etc., one constituent can be set at the critical point of adsorption and will not interfere with the simultaneous separation of the second constituent e.g. according to its size.

Theoretically, LCCC presents a powerful and attractive tool for characterization of complicated polymer systems such as certain copolymers, polymer mixtures and functionalized oligomers. Consequently, numerous papers have appeared recently in which the LCCC principle had been applied succesfully either alone or in combination with other LC procedures ("multidimensional chromatography") and/or with nonchromatographic methods of analysis (e.g. MALDI-TOF). However, a more profound analysis of the LCCC experimental feasibility leads to the queries as to the precision and repeatability of method, especially for high molar mass polymers.

We shall discuss several experimental problems connected with LCCC
- identification of critical conditions
- excessive sensitivity of critical conditions to eluent composition, to column packing parameters, to temperature, pressure and flow rate, to physical structure of macromolecules of the same chemical composition e.g. to their

tereoregularity, as well as to the slight defects in the chemical composition of macromolecules

- role of preferential interactions among column packing, mixed eluent components and dissolved macromolecules
- limited recovery in the higher molar mass area
- selective and overall detection of constituents of macromolecules in effluent
- additional peak broadening and the mutual influence of the incompatible chains in the interpolymers
- excessive sample dilution in the case of bi- or multidimensional liquid chromatographic procedures.

Above problems set the contemporary experimental limits to LCCC and warn of the unjustified optimism concerning the applicability of method to high polymers. On the other hand, these problems present also a challenge for further research directed towards improvements in the LCCC methodology.

EXPERIENCES WITH INTERLABORATORY GPC-EXPERIMENTS

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Summary

On the base of some round robin experiments organized in the last years by a DIN working group and by the IUPAC working party IV.2.2, the paper will discuss measures for getting a better interlaboratory reproducibility of GPC results. The most important points are:

- selecting the columns according to their separation efficency
- avoiding artifacts in the distribution curves, caused by gaps in the pore volume distribution
- avoiding adsorption and memory effects
- calibration: quality of calibration standards suitable selection of calibration points influence of injection concentration
- selecting a common detector
- data treatment, especially finding the correct baseline
- detailed data report.

STUDY OF POLYSTYRENE-BLOCK-POLY(METHYL METHACRYLATE) MICELLES BY SIZE EXCLUSION CHROMATOGRAPHY / LOW ANGLE LASER LIGHT SCATTERING

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Block copolymers in selective solvents (i.e. thermodynamically good solvent for one block and at the same time poor solvent for the other block) form multimolecular associates - called micelles - having a core formed by block of low solubility and a protective shell formed by block of high solubility. Micelles are formed via so-called closed association, which is characterized by an equilibrium between micelles (M), with a narrow molecular-weight and size distribution, and the molecularly dissolved copolymer - unimer (U):

$$nU \overset{K_m}{\Longleftrightarrow} M \tag{1}$$

where n is the association number and K_m is the micellization equilibrium constant. Size exclusion chromatography (SEC) is known to be a powerful method of characterizing polymer molecules in solution based on the separation of macromolecules according to the hydrodynamic volume. However, the application of SEC to the characterization of individual components for a micellar system is not straightforward: the separation of micelles from unimer in a SEC column causes continuous disturbing and subsequent re-establishment of the above equilibrium. (1) Thus, the shape of chromatograms strongly depends on the relative rates of SEC separation and of the $U \Leftrightarrow M$ association and dissociation and it is therefore evident that SEC can provide (quantitative) information on the dynamics of the $U \Leftrightarrow M$ equilibrium.

An example of SEC results obtained for micellar system poly(styrene-b-methyl methacrylate) diblock copolymer in a mixed solvent, 1,4-dioxane/cyclohexane is reported. Good separation of peaks of micelles achieved for this system enabled a direct molecular-weight characterization of micelles by low angle laser light scattering (LALLS) detector. Experiments with changing flow rate and concentration of injected sample solution showed moderately fast unimer-micelles re-equilibration in the course of separation. The application of LALLS detector was found to be very advantageous for a better interpretation of experimental elution curves.

CHARACTERISATION OF STIFF MACROMOLECULES BY MULTIDETECTION GPC

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Due to the recent work of Tsukahara (1) methacryloyl-endfunctionalized polystyrene macromonomers can be readily homopolymerized to high degrees of polymerization (Pn = 500-1000) provided the macromonomer molar mass is below Mn = 10000 g/mol. At first it was believed that the structure of the molecules resembled a flexible comb. However, GPC investigations utilizing a multi-angle light scattering detector reveal a "bottlebrush"-like conformation (2,3) with a Kuhn statistical segment length of the MMA main chain of lk = 1000-2000 A depending on the molar mass of the macromonomer which was determined by MALDI/TOF-MS. Thus an easy route to prepare rod-like macromolecules consisting of "commodity" monomers is established. Small angle x-ray scattering on concentrated solutions indeed demonstrate the expected existence of lyotropic liquid crystalline phases which are also observed by a polarizing microscope for thin films (4).

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NEW TRENDS IN THE SIZE-EXCLUSION CHROMATOGRAPHY OF POLYMERS: THEORY AND APPLICATIONS

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The advances in the GPC theory and some new applications are considered.

The universal relationship between the distribution coefficient and the slope of a calibration curve has been obtained from the GPC theory and experiments. Based on this fact, the concept of an "internal standard" in the polymer GPC has been advanced which provides the possibility of polymer polydispersity determination with carrying out no calibration procedure. The new method of polymer polydispersity determination needs the chromatogram of the polymer under investigation only.

The experimental corroboration of this method has been provided using various mixtures of polymer standards.

The proposed method is believed to be beneficial in studies of new polymers where standards are not available.

The resolution obtainable with the GPC method in the macromolecule and globular protein separations has been analyzed from the standpoint of the theory. A relationship between optimal conditions for the separation and the structure of porous material has been established. An influence of the thermodynamic quality of the solvent upon the resolution of polymers in GPC is discussed.

CHARACTERIZATION OF POLYETHERS BY LCCC, LAC, AND SEC

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In the characterization of polyethers, at least two distributions have to be taken into account: the molar wass distribution (MMD) and the functionality type distribution (FTD). Additionally, the distribution of chemical composition (CCD) will be important in the case of copolymers.

The MMD is typically determined by Size Exclusion Chromatography (SEC), which, however, is not capable of separating even higher oligomers. Moreover, the calibration lines may be considerably different for different polymers, and sometimes even for different functionalities of the same polymer. It has also to be taken into account, that detector response will depend on molar mass and chemical composition of a polymer chain. Hence it will be advantageous to use SEC rather as the second dimension in a two-dimensional separation, in which the first dimension should separate according to functionality or chemical composition.

This can be achieved by Liquid Chromatography. under Critical Conditions (LCCC), in which separation occurs exusively according to functional groups (or one block of a copolymer), while the main chain (or the other block) become chromatographically invisible.

The pure polymer homologous series thus obtained can be analyzed by subsequent SEC to yuid a three-dimensional map of the polymer. As the separation power of SEC is limited, one may achieve a much better resolution by Liquid Adsorption Chromatography (LAC) or Supercritical Fluid Chromatography (SFC), which separates to similar criteria. Depending on the nature of the repeating unit and the end groups, one has to apply normal or reversed phase conditions. In this way, a complete separation of all oligomers can be achieved even for higher degrees of polymerization..

CHROMATOGRAPHY OF NON LINEAR BLOCK COPOLYMERS

N. Hadjichristidis

CHARACTERIZATION OF CHEMICAL HETEROGENEITY BY CONVENTIONAL GPC

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Introduction:

This paper describes a reliable and rapid method for the analysis of copolymers by size-exclusion chromatography (GPC). The properties of copolymers mainly depend on the choice of comonomers, molecular weight and composition. In the case of homo-polymers the molecular weight distribution determines many important properties. Additionally, the knowledge of composition distribution for copolymers is most important, since it influences physical properties, e.g. chain dimensions and rheological data, to a great extend.
The combination of multiple concentration detectors alone allows the direct measurement of chemical composition distribution along with copolymer molar masses for each GPC slice.

Theory:

GPC separation of copolymers is generally more complex than GPC characterization of homo-polymers. This is due to the fact that a copolymer shows a molar mass distribution as well as a comonomer distribution. Since a polymer is separated according to molecular size, GPC may yield fractions that can be polydisperse in molar mass *and* chemical composition. For copolymers of styrene and MMA of the same molar mass, retention increases in the following order: poly(styrene-r-MMA), homo-polystyrene, poly(styrene-b-MMA) and homo-PMMA[1].

Copolymer analysis using the PSS GPC software package is based on a modified multi-detection method first reported by Runyon et al.[2]. Molecular weight and composition information is obtained in the same GPC run without any special sample preparation necessary.

Molar masses of the copolymers are accurate if segment-segment interactions are negligible as was shown by Benoit et al.[3]. The precision of the compositional information is not affected by polymer topology, however. Deviations in the comonomer ratio are only possible, if the detected property is dependent on the environment. This is the case if neighbor-group effects exist. However, the possibility of electronic interactions causing such deviations is very low, since there are too many chemical bonds involved. For other types of interactions (e.g. charge-transfer) it is an effect to keep in mind.

Even no specific calibration is needed if the copolymers have block structures[4]. Different copolymer topologies like comb-shaped or star-shaped polymers require special calibration or further characterization (e.g. by on-line MALLS or viscometry) in order to give accurate molecular weights.

Chemical composition of copolymers is measured by calibrating the detectors, i, for all comonomers, k. The detector response factor, f_{ik}, is directly calculated from the detector output, U_i, and the injected mass of the corresponding homopolymer. The absolute concentration w_{ik} is then given by:

$$U_i(V) = \Sigma f_{ik} \cdot w_{ik}(V)$$

Copolymer molar masses, M_c, are calculated following Runyon's[3] findings:

$$\lg M_c = \Sigma\, w_k \cdot M_k$$

Results

The validity of copolymer composition determined by multiple detection was checked by the analysis of block copolymers consisting of styrene and methyl methacrylate segments (cf. table 1) as well as by statistical copolymers consisting of MMA and t-butyl methacrylate using NMR-measurements, gravimetry and element analysis. The precision of molar masses calculated for block copolymers are checked by on-line multi-angle laser light scattering. The copolymer GPC analysis of a 4-arm poly(styrene-b-butadiene) copolymer by multiple detection shows homogeneous butadiene content throughout the complete distribution of the blcok structure. GPC measures 65% (w/w) diene, as compared to 67% in the comonomer feed. A homopolymer contamination can be found at low molar mass. Since this small peak contains no butadiene, it can be attributed to the polystyrene precursor.

A comparison of these results with on-line MALLS demonstrates the validity of the assumptions mentioned above. GPC-MALLS reports 154000 D and 320000 D for M_n and M_w, respectively, copolymer GPC with multiple concentration detectors 151000 D and 294000 D. The molar mass of a single arm is determined by MALLS as 92000 as compared to 93000 D by copolymer GPC.

The the calculated copolymer calibration curve agrees very well with the one measured by on-line MALLS. A good agreement is found between calculated and measured copolymer molecular weights apart from the very high and low molar masses, where polymer concentration is very low.

It will be shown that the GPC copolymer characterization can be used efficiently to elucidate polymerization processes. Several examples deal with block copolymer syntheses via anionic polymerization techniques.

Other types of segmented copolymers, e.g. comb-shaped polymers, also illustrate the usefulness of composition distribution and molecular weight information in order to get a better understanding of structure-property relationships of copolymers.

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GPC MEASUREMENTS OF PHENOL-FORMALDEHYDE RESING USING POLYDISPERSE PORE SIZE DISTRIBUTION POLYSTYRENE GEL COLUMN AND SODIUM TRIFLUOROACETATE MODIFIED TETRAHYDROFURAN

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Summary

GPC measurement method of phenol formaldehyde novolac resin(PFNR) by use of broad and smooth pore size distribution polystyrene gel column and tetrahydrofuran(THF) modified with sodium trifluoroacetate(STFA) was developed. We carried out the investigation using GPC-LALLS(low angle laser light scattering) and GPC-Visc(viscometry) for the estimation of the elution behavior of the polymer molecules in the column and the determination of the experimental parameters. A specially designed broad and smooth pore size distribution gel column was found to overcome the problem of the rugged shape or concave-convex shape on the GPC chromatograms due to the complicated shape of the pore size distribution of polystyrene gel or the complicated shape of the calibration curve which is observed if the conventional column combination of a different exclusion limit or the mixed bed column should be used. The problem of abnormal tailing to the short elution time caused mainly by the THF solvation and the repulsion after the adsorption of PFNR molecules by gel was overcome by the addition of 0.1 (W/V)% of STFA in THF. The GPC method proposed provides a reasonable GPC curve and has good reproducibility.

Introduction

Since the advent of GPC, the method has been investigated and applied to measure molecular weight distributions(MWD) of phenol formaldehyde resins(PFR). Usually the GPC measurements of MWD of PFR may be carried out using polystyrene gel column with THF[1-4] or DMF[5] with salt. However, in the measurements under these conditions, we may encounter two problems which lead to the erroneous results of MWD. First, if the sample has a broad MWD and a conventional column combination with different exclusion limits or if the mixed bed column is used, an abnormal rugged

shape or concave-convex shape appears in the GPC curve due to the rugged
shape of the pore size distribution or the complex calibration curve of
the gel combination. Second, in the measurement using THF as a solvent, an
abnormal tailing to the short elution time reaching even   exclusion
limit is observed. The problems are associated with the complex property
of PFR and the nature of column packing gel. Recently the GPC method
after acetylation of OH groups in the sample was reported[6].

Experimental

Commercial PFNR "Sumilite resin" PR-50731(Sumitomo Bakelite) was used as
a sample. The ortho/para ratio(1.35/1.00) was determined by IR. The GPC-
LALLS(150C;Waters,KMX-6;Chromatix) and GPC-Visc(150C;Waters,520a;Visco-
tek) were used for the determination of the absolute molecular weight
and the intrinsic viscosity at each elution time of GPC. Three TSK-GEL-
Linear-M columns,7.8mm.d.-30cm.l.(specially ordered by author and made
from TOSO Co.,not commercialized) were used.

Results and Discussion

The sample"Sumilite resin" showed an abnormal rugged shape or concave-
convex shape in the GPC curve under the experiment using a conventional
column combination of different exclusion limits or the mixed bed column.
The sample also showed an abnormal tailing to the short elution time
when pure THF was used with any polystyrene gel colums. A phenomenon of
rugged shape was not observed under the measurement using a limited ex-
clusion column. Therefore,we estimated that the rugged shape in GPC
curve did not show the true MWD shape of the sample but it was a kind of
the artifact .due to the rugged shape of the pore size distribution of
the gel. Then the column design was made to get good GPC curves. Special-
ly designed TSK-GEL-Linear-M column which has the broad-flat-smooth pore
size distribution was used to find that it provides smooth GPC curves
without any rugged shape. GPC-LALLS and GPC-Visc results at various STFA
concentration suggested that the abnormal tailing is due to the THF sol-
vation and the repulsion after the adsorption of the sample to the poly-
styrene gel. We found that STFA has the effect of supressing the adsorp-
tion of the sample, THF containing 0.1 wt% of STFA is the best for the
GPC elution performance, STFA is soluble as high as 3 wt% in THF, and
the mixed solvent does not degrade the polystyrene gel column.

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THEORETICAL AND PRACTICAL ASPECTS OF GRADIENT POLYMER ELUTION CHROMATOGRAPHY

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Introduction

Gradient Polymer Elution Chromatography (GPEC) appears to be a powerful technique in the determination of molecular mass distribution and chemical composition distribution. The former application is mainly used for low molecular mass homopolymers, whereas the latter is particularly suitable for high molecular mass copolymers.
The technique is based on precipitation and selectively redissolving polymers by means of a non-solvent solvent gradient. Differences in solubility, caused by molecular mass or copolymer polarity are reflected in different retention volumes during the GPEC experiment.

Solvent non-solvent combinations

Turbidimetric titrations appear to be a good technique to explore solvent non-solvent combinations suitable for GPEC experiments. GPEC eluents can be sorted according to their polarity. If then the solubility of well defined polymers in these eluents is tested, there appears to be a polar and a non-polar transition from solvent to non-solvent (or vice versa). Similarly if solvent non-solvent mixtures are used, it appears that the ratio of solvent to non-solvent required to reach the onset of turbidity (demixing) is related to the polarity of the separate components in the system (polymer, solvent, and non-solvent). When Hildebrand parameters are calculated from the fractions of solvent and non-solvent at the onset of turbidity, the variations among different combinations are limited. The same is observed for the critical solvent composition (CSC) on a given stationary phase for different solvent non-solvent combinations. What we do find in the case of CSC is that the maximum molecular mass of the polymer soluble at the CSC strongly depends on the specific solvent non-solvent combination. For example, polystyrene with a molecular mass $M > 35,000$ appears to be insoluble in the CSC of THF/H_2O on C18 modified silica, whereas $M = 240,000$ is still soluble in the CSC of dichloromethane/acetonitrile and of THF/acetonitrile.

Molecular mass dependence

As indicated above, GPEC may be used to determine molecular mass distributions of low molecular mass polymers. Glöckner has previously shown that if the fraction of solvent in the eluens is plotted versus the reciprocal of the square root of the molecular mass, straight lines are obtained. We found that deviations from this straight line occur in many cases. The curves for different polymers have a similar shape, but the extent to which they deviate from a straight line may differ quite significantly. It is clear that the present theory to describe the separation mechanism in GPEC is incomplete. We are presently trying to study solubility aspects and exclusion/adsorption aspects separately.

As an example of the separation in GPEC, two chromatograms of an aromatic polyester are given below. The top one is a GPC measurement, and the bottom one a GPEC measurement. It is clear that the extent to which oligomers are separated is much higher in GPEC than it is in GPC.
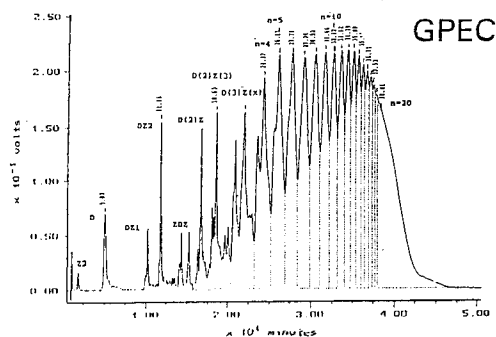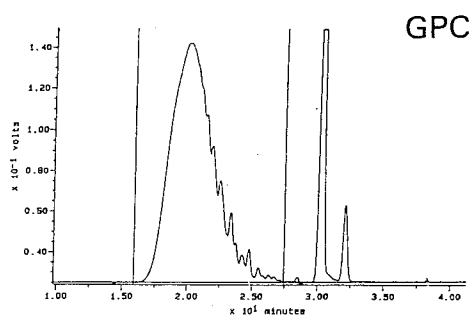
NEW ASPECTS OF DETERMINATION OF POLYMER HETEROGENEITY BY 2-DIMENSIONAL ORTHOGONAL LIQUID CHROMATOGRAPHY AND MALDI-TOF-MS

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Liquid Adsorption Chromatography at Critical Conditions (LACCC) in coupling with Size Exclusion Chromatography (SEC) has the potential to discribe different kinds of polymer heterogeneity. Beside determination of „critical" conditions of samples with identical structure, but different molar masses the main problems are the chemical identification and structural characterization of in LACCC-mode separated peaks. New aspects in this field are given by using spectroscopic structure detection (FTIR, UV-diode array, NMR) or reaction detection (post column). Optimization of determination of polymer heterogeneity is possible by using Matrix-Assisted Time-of-Flight Mass Spectrometry (MALDI-TOF-MS). This method is helpful for characterization of peaks, separated by LACCC according to structure unit ,endgroups and for SEC calibration.

Examples of LACCC applications concern problems of branching, modelling of degradation kinetics and relations between composition of mobile phase and polymer structure.

The characterization of separated peaks was done by using MALDI-TOF-MS, reaction detection, UV-diode array detection, Evaporative Light Scattering Detection (ELSD) and coupling with SEC.

Investigations concern :
⇒ polyesters / structure - LACCC- retention
⇒ thermal degradation of polylactides
⇒ polyethers / branching
⇒ polysiloxanes / characterization

Problems in connection with quantification will be discussed.

CHROMATOGRAPHIC CHARACTERIZATION OF POLYMERS AND ANTIOXIDANTS IN THERMOPLASTICS BASED ON OLEFINS

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Thermoplastics based on polyolefins are the most important synthetic polymers with respect to the volume of production. Their behaviour in processing and application is strongly dependent on their molecular architecture, e.g. the type of branching, and their polydispersity, but also on the influence of the content of additives, especially of antioxidants. All these molecular features can be unravelled by chromatographic methods.

The state of the art of these methods will be reviewed with main emphasis on size exclusion chromatography coupled with low laser light scattering applied to polypropylene homopolymer[1,2] and linear polyethylene in the full range from very low to high density grades[3]. The influence of peak broadening on the evaluation of molar mass distribution[4] and a method for extension of the measuring range to higher molar mass[5] will be discussed. Finally, a new method of sample preparation and new separation systems for the HPLC of antioxidants will be described[6].

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APPLICATIONS OF MULTIDETECTION IN THE GEL PERMEATION CHROMATOGRAPHY ANALYSIS OF COMPLEX POLYMERS

J. Lesec and M. Millequant

EXAMINATION OF THE CHEMICAL AND PHYSICAL COMPOSITION OF POLYOLEFINE COPOLYMERS

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The numerous applications of polyethylene and poly-propylene polymers demand a proper design of the properties to be successfull on the market. The origin of the application properties is founded in the molecular chain structure. To attain higher performance most polymers in this field are not only homopolymers, but are copolymers of 1-alkenes with ethylene or propylene. Therefore it is important not only to control the molecular mass (M_w) and the molecular mass distribution (MMD) but also to control the chemical composition (CC) and the chemical composition distribution (CCD).

For the analysis of the polyolefines high-temperature methods were developed many years ago. On the analytical scale HT-SEC-techniques are well developed for the analysis of the MMD. On the preparative scale the solvent/nonsolvent fractionation is still of value to obtain resonable amounts of material for further analysis.

The analysis of the CCD of polyolefines by Temperature Rising Elution Fractionation (TREF) has become a standard method within the last decade. The method relies the change of the melting point of polyolefin-copolymers with comonomer content, which can be described by the Flory-Vrij-Theory of semicrystalline polymers.

Examples of application of these methods for the analysis of polyolefine copolymers are shown and relations to product properties are drawn. The advent of the new metallocene catalysts allows the production of homogenous polyolefines with unprecedented properties, which can clearly be explained by the results of these analytical methods.

With the combination of the TREF and HT-SEC a complete composition distribution analysis of the polymers is accessable. The results are condensed in a 3D-Diagramm, which shows the impact of the catalyst system, production process and gives an estimate of the property profile. The information can be used successfully for the design of new products with optimized properties.

SEC OF POLYMERS: MAIN LIMITATIONS AND SOME DATA
TREATMENT SOLUTIONS

G. Meira

CANCELLED

CONDUCTING POLYMERS, POLY(3-ALKYLTHIOPHENES), CHARACTERIZATION BY MEANS LIGHT SCATTERING AND VISCOMETER ON-LINE SEC DETECTORS

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Conventional SEC using a concentration detector, as a differential refractometer, is often hindered by the problem of appropriate calibration for polymers of different structure. The availability of on-line absolute detectors, as a Light Scattering and/or a Viscometer, overcomes the calibration problem. Beside the multidetectors SEC system greatly increases the information content that can be obtained. On-line viscometer, VIS, used in conjunction with a concentration detector allows to obtain the experimental intrinsic viscosity distribution, IVD, and indirectly the molecular weight distribution, MWD, from the universal calibration. On-line Multi Angle Laser Light Scattering, MALLS, allows to obtain true molecular weight without calibration and the root mean square radius, $<s^2>^{\frac{1}{2}}$, of every fraction of the sample. If a sufficient range of molecular weight, intrinsic viscosity $[\eta]$, $<s^2>^{\frac{1}{2}}$ is present in the samples from the experimental $<s^2>^{\frac{1}{2}}=f(M)$ and $[\eta]=f(M)$ relationships it is possible to study the molecular conformation. However several experimental conditions strongly influence the accuracy these studies.

Poly(3-alkylthiophenes), P3AT, have been the subjects of many researches. The addition of alkyl side chains, with a number of carbon atoms higher than four to Polythiophene main chain renders the polymers soluble while preserving their conductivity. Furthermore P3AT's solubility allows the use of traditional solution characterization techniques. P3AT have been widely characterized by means conventional SEC relative to Polystyrene calibration in THF or Chloroform solvent. In this study we present a SEC-VIS-MALLS characterization of P3AT polymers by means three on-line detectors system: MALLS, Viscometer and concentration detector. The characterization regards several samples of Poly(3-n-alkylthiophenes) where n-alkyl is 'butyl, hexyl, octyl and decyl.

CRYSTAF: CRYSTALLIZATION ANALYSIS FRACTIONATION. A NEW APPROACH TO THE COMPOSITION ANALYSIS OF SEMICRYSTALLINE POLYMERS

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Crystallization Analysis Fractionation is a new technique for the analysis of composition distribution in semicrystalline polymers; more specifically for the analysis of branching distribution in Polyethylene and tacticity in Polypropylene type resins.

CRYSTAF as well as TREF (Temperature Rising Elution Fractionation) are separation techniques which fractionate species of differing crystallizability by slow cooling of a polymer solution. TREF however, demands in addition to the crystallization step a second temperature cycle, elution step, to obtain information on polymer composition. CRYSTAF, on the other hand, extracts the information in the crystallization cycle by monitoring the solution concentration depression as temperature goes down; thus reducing significantly the analysis time and symplifiyng the hardware needs.
The simultaneous analysis of five samples is carried out in less than six hours.

CRYSTAF principles are presented and the following applications are described :

Metallocene type polyolefins
Narrow composition distribution curves with crystallization peak temperatures having a linear relationship with amount of comonomer incorporated.

Polyolefines Blend analysis

Linear Low Density Polyethylenes
Analysis of the homopolymer fraction and remaining soluble fraction at the lowest crystallization temperature.

Polypropylenes
Tacticity analysis: iso, syndio and atactic fraction percentages.
Presence of ethylene comonomer.

CHARACTERIZATION OF POLYMERS BY MALDI-TOF AND GPC MOLECULAR WEIGHTS ESTIMATES IN SAMPLES OF VARYING POLYDISPERSITY

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Matrix Assisted Laser Desorption Ionization - Time of Fligth Mass Spectrometry (MALDI-TOF MS) allows detection of large molecules such as those present in synthetic and natural macromolecules.

Synthetic polymers may show a wide range of molecular weights distribution (MWD), according to the synthetic method used in their preparation. We have studied several Polymethylmetacrylate (PMMA), Polystyrene (PS), Polyethyleneglycol (PEG) samples with varying MWD, and also a number of condensation polymers such as Nylon 6, Polycarbonate and Polyesters. Measurements of MALDI-TOF spectra in linear mode were used in our work to estimate the MW and MWD of the polymer samples. Our results show that the molecular weight estimates provided by MALDI-TOF measurements agree with the values obtained by conventional techniques such as GPC, only in the case of polymer samples with very narrow MWD.

However, when the polydispersion reaches values around 1.10 the difference between the MW measured by GPC and MALDI may amount up to about 20%. At higher dispersions, the MALDI spectra fail to yield reliable MW values.

GPC AND SOME EMERGING TECHNIQUES IN POLYMER MWD CHARACTERIZATION

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Polymer chain length is probably the most important single parameter which controls the end-use properties of plastic materials. Molecular weight and molecular weight distribution (MWD) are routinely determined by Gel Permeation Chromatography (GPC). Benefiting from 30 years of continuous development, GPC has evolved as a state-of-the-art technique in MWD analysis. There are, however, a few situations for which GPC is inadequate or inefficient for characterization purpose.

1) *MWD of insoluble thermoplastics*

Being a solution characterization technique, GPC requires prior dissolution of the polymer. The development of «high performance» engineering thermoplastics has generated a special class of materials with remarkable solvent resistance. Dissolution of these compounds generally requires original solvent mixtures at a temperature close to the polymer melting point. Although some modern GPC instruments can perform at temperatures as high as 200°C, analysis under such extreme conditions has always been delicate. Columns lifetime is considerably shortened and thermal degradation difficult to be avoided. For most of the high temperature thermoplastics as well as the fluorinated polymers, the best characterization medium would be the bulk state. Recent efforts have been focused on predicting MW and MWD from the dynamic viscoelastic properties of the polymer melt [1]. One technique, based on the change in the storage modulus (G') with the sollicitation frequency (ω), has been applied in our laboratory. By comparing the rheological results with the GPC data on sulfonated PEEK samples, it is determined that the technique could be used for the MWD determination. The main difficulty, thermally induced crosslinking reactions, could be minimized by selecting a sufficiently low test temperature ($\leq$ 360°C).

2) *MWD of very high and very low MW polymers (pleistomers and pleinomers)*

Synthetic polymers were traditionally used for engineering purpose. As a compromise between engineering and processing properties, the MW range of commercial polymers was generally limited to 10^4-10^6 dalton. Fortunately, it is also within that range that GPC is the most effective in MW characterization. During the last decade, new applications have extended the useful MW range of synthetic polymers. In the upper limit, ultra-high MW polymers are of increasing importance in plasma polymerization, in the production of oriented fibers and of scratch/wear resistant materials. In the opposite MW scale, functionalized polymers and oligomers are increasingly used in a variety of specialty applications ranging from additives, adhesives to optoelectronics. GPC characterization beyond the

conventional MW range can give rise to considerable experimental difficulties as a result of shear-induced degradation, limited resolution and lack of proper calibration procedure. Studies performed in our laboratory [2] and elsewhere [3,4] suggested that the technique of flow birefringence could be applied for computing the MWD of polymers above 10^6 dalton. For low MW polymers and copolymers, on the other hand, the technique of matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry with its unique capabilities to resolve individual oligomers within a MWD, proves to be the best alternative to GPC characterization [5].

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THE GENERALIZED APPROACH OF FRACTIONATION OF NON-TRADITIONAL MACROMOLECULES BY SIZE-EXCLUSION CHROMATOGRAPHY

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Size-exclusion chromatography (SEC) is high selective, high sensitive, and high speed method of polymer fractionation according to their sizes. For the linear flexible chain polymers (traditional objects) the theoretical substantiation of the method and the principles of its practical application are well- known. But most interesting and difficult problem is the development of this method to investigate non-traditional macromolecular objects, the retention volume of which depends not only on the molecular weight of the macromolecules but also upon the other their characteristic parameters. These characteristic parameters are: composition (for block, random, graft copolymers), charge density (for polyelectrolytes), degree of branching (for long-branched polymers), degradation index (for giant macromolecules). Special interest of non-traditional situations are: a) investigation of the macromolecules by SEC in vacancy mode, when the solvent is chromatographed in solution of polymer as the eluent, b) SEC in continuous porous media, when intraparticle and interparticle diffusion are absent, c) SEC in the conditions of gradient of thermodynamic potential, when diffusion transport will facilitate and internal porous structure (fractal gauge) can be changed.

The solution of the problem enable us to work out generalized conception of optimum fractionation of these complex macromolecular objects by SEC with receiving precise molecular and other characteristics.

LIQUID CHROMATOGRAPHY AT THE CRITICAL POINT OF ADSORPTION - A NEW TECHNIQUE FOR POLYMER CHARACTERIZATION

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The molecular heterogeneity of complex polymers is determined by the chemical composition, the sequence length of the components in the polymer chain, the functionality and the molar mass distribution. Typically, polymer analysis has to deal with the superposition of different distributions, e.g. molar mass distribution and functionality type distribution in the case of telechelics, and therefore, size exclusion (SEC) and liquid adsorption (LAC) chromatography alone can not completely determine the molecular heterogeneity.

A new chromatographic method - liquid chromatography at the critical point of adsorption - is operating at the transition point from SEC to LAC. At this critical point of adsorption, the molar mass dependence of the retention time disappears, and separation is solely accomplished with respect to functionality or chemical composition.

The application of different modes of liquid chromatography, including chromatography at the critical point of adsorption (CC), to the analysis of complex polymers will be discussed in the paper. It will be shown that block copolymers can be analyzed with respect to the block lengths of the individual blocks by chromatography at the critical point. A complete description of the molecular heterogeneity of block copolymers is possible using two-dimensional chromatography CC vs. SEC. Binary polymer blends can be separated with respect to the components regardless of their individual molar masses under experimental conditions, where one component elutes at the critical point of adsorption. It will be shown that even very similar polymers, such as poly(n-butyl methacrylate) and poly(t-butyl methacrylate), can be completely separated.

In addition to sophisticated separation techniques, the application of selective detection systems in liquid chromatography of complex polymers is of great importance. First results on the application of FTIR, viscometric, and MALDI mass spectrometric detectors in liquid chromatography at the critical point of adsorption will be discussed.

THE USE OF GPC-MALLS FOR THE INVESTIGATION OF BRANCHING

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Gel permeation chromatography (GPC) coupled with the multiangle laser light scattering (MALLS) photometer has been applied to the analysis of several branched polymers. Attention has been paid to the interpretation of experimental data. The ability of particular methods to identify and characterize branched polymers has been discussed.

The branched molecules can be formed by polycondensation using a mixture of two and more functional monomers; radical copolymerization of vinyl and divinyl monomer mixtures; group transfer polymerization; crosslinking of linear polymers by chemical reaction, heat treatment or irradiation; radical polymerization with chain transfer to monomer or polymer. Branched molecules can often appear undesirably as a result of various side reactions (addition of glycols and water on double bonds of unsaturated acids during manufacture of unsaturated polyester resins; random chain transfer to polymer or monomer during synthesis of linear polymers; polymerization of bifunctional monomers containing polyfunctional impurities; ageing).

The branching significantly affects mechanical properties, solubility, chemical resistance, viscosity or curing behaviour. These effects can be both - positive or negative - depending on circumstances and polymer application. Therefore, it is important to have a powerful analytical tool capable of branching investigation. At constant molecular weight the root mean square (RMS) radius (radius of gyration) decreases with the increasing degree of branching. Thus the comparison of RMS radii and molecular weights of a series of samples can give information on the degree of branching. However, application of classical light scattering, which yields both molecular weight and RMS radius, is limited to polymers with a narrow molecular weight distribution because in samples with a broad molecular weight distribution the z-average RMS radius is more affected by high-molecular-weight branched fractions than the weight-average molecular weight. Therefore the combination of GPC as a method for fractionation with light scattering may be a suitable approach.

The investigation of branching can be separated into two different analytical tasks:
1. Characterization of branched polymers purposefully prepared by special synthetic methods, i.e. the determination of RMS radius distribution and RMS radius moments, and relations between RMS radius, branching ratio, number of branches per molecule and molecular weight. Determination of linear fractions in branched polymer.
2. Identification of undesirable branched species in linear polymers.

There are three plots that can be used for the investigation of branching by GPC-MALLS. They are:
1. RMS radius versus molecular weight plot (conformation plot).
2. Molecular weight versus elution volume plot (calibration curve).
3. Intrinsic viscosity versus molecular weight plot.

RMS Radius vs. Molecular Weight Plot

The slope of the RMS radius vs. molecular weight plot gives information about conformation of polymer chain. Typical values for linear random coils lie between 0.5 and 0.6, while lower values indicate the presence of branched molecules (0.33 for spheres). A slope higher than 0.6 are indicative of rod-like polymer molecules (1 for rods). The value of the slope may be influenced by a way of the treatment of experimental data (Zimm or Debye extrapolation).

There are several factors that can influence the plot:
In a randomly branched polymer or in a mixture of linear and branched polymer each volume slice may contain species of the same hydrodynamic volume but different molecular weight, and degree and architecture of branching. Thus GPC-MALLS relates the weight-average molecular weights with the z-average RMS radii.

Separation other than size exclusion may retain some molecules which elute at higher elution volumes than it would correspond to their hydrodynamic volume. Due to this effect, the slices of higher elution volume contain a mixture of molecules of lower molecular weight (separated by pure size exclusion) and molecules of higher molecular weight which were retained by nonsize exclusion mechanism.

The decrease of the slope due to the branching can be eliminated by other effects leading to the extension of polymer chain. An analysis of polyelectrolytes is a typical example.

Molecular Weight vs. Elution Volume Plot

Molecular weight vs. elution volume plots (calibration curves) of linear chemically identical samples must overlap and any deviation proves branching.

Intrinsic Viscosity vs. Molecular Weight Plot

The intrinsic viscosity is a sensitive measure of the volume occupied by a polymer molecule in a dilute solution. Hence, the intrinsic viscosity vs. molecular weight plot is another possibility of the characterization of branched polymers by GPC-MALLS. Since the MALLS detector measures molecular weight for each volume slice, the intrinsic viscosity can be calculated from the universal calibration. The essential condition for this procedure is that the sample under investigation separates by pure size exclusion and obey the universal calibration.

An advantage of molecular weight vs. volume and intrinsic viscosity vs. molecular weight plots compared with the relation between the RMS radius and molecular weight is that they can be used also in the range of lower molecular weights where the determination of RMS radius is uncertain or impossible.

Conclusions

GPC-MALLS can be used for the identification of the presence of branched species in linear polymers as well as for the characterization of branched polymer samples. Information about branching can be inferred from the relationships RMS radius - molecular weight, molecular weight - elution volume and intrinsic viscosity - molecular weight. Experimental data must be carefully evaluated with respect to a possible nonsize exclusion separation, repulsive forces, and the influence of the way of light scattering data extrapolation on RMS radii.

SEPARATION OF COPOLYMERS BY ADSORPTION CHROMATOGRAPHY

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Introduction

Chemical composition distribution (CCD) has been analyzed by cross fractionation or thin layer chromatography. However, the former method requires a long time for separating the sample and determining the composition of each fraction. It is difficult to quantitatively determine CCD by the latter method due to poor resolution. Recently, HPLC has been utilized to analyze CCDs of styrene-methyl methacrylate (St-MMA)[1], St-butadiene (St-BD)[2,3], BD-acrylonitrile (BD-AN)[4], and other copolymers[5].

In this report, random and graft BD-St copolymers and St-MMA-AN terpolymers were separated by HPLC. The separation mechanism was also discussed.

Experimental

Random St-BD copolymers were prepared using benzoyl peroxide as an initiator in bulk. Poly(BD-graft-St) samples were prepared by lithiating cis-polybutadine with butyl lithium in the presence of N,N,N',N'-tetramethyl ethylene diamine followed by adding St monomer. St-MMA-AN copolymers were prepared with a radical initiator.

Packing materials for HPLC column were prepared by suspension or seed polymerization. Columns were packed by a slurry method. HPLC was conducted using two pumps with a linear. UV or evaporative mass detector was used for monitoring the effluent.

Results and Discussion

St-BD random copolymer can be separated by normal phase (NP) HPLC using acrylamide (AA) gel and n-hexane/chloroform eluent with the gradient elution of increasing acrylamide content. Samples eluted out in the order of increasing St content. It is confirmed that these samples are separated not by phase separation but by adsorption mechanism comparing the cloud point and the composition of eluent.

Poly(BD-graft-St) samples with different St contents and graft lengths were prepared using an anionic initiator. In Figure 1 elution times of the graft polymers were plotted against St content together with those of random copolymers. The elution of the graft copolymer retarded with the increase of graft length and of St content. In the case of block copolymer, the elution time also became longer as the block length of St unit increased[3]. This contrasts with the results of poly(MMA-graft-St)[6], which showed no effect of graft length on the elution volume.

St-BD copolymer was also separated by reversed phase (RP) HPLC using less polar St gel and with acetonitrile/chloroform eluent. The samples were separated by phase separation mechanism. This may be attributed to the fact that the stationary phase is more polar than the sample.

In the case of the separation of the copolymer by adsorption mechanism, the separation is mainly governed by the relationship among the polarities of eluent, gel, and the sample. For NP HPLC, sample eluted from the less polar molecules and in the opposite order for RP HPLC. In the experiment of separation of St-butyl methacrylate copolymers which are consisting of monomers having almost the same polarity, the specific interaction between the sample and the gel or the eluent also played an important role[7,8]. The elution order differs by changing the type of eluent even using the same stationary phase or by changing the type of column with the same eluent. This finding indicates the possibility of the separation of terpolymers by composition by cross-fractionation using two types of HPLC.

Figure 2 shows the elution time of St-MMA-AN terpolymer for NP HPLC using AN or

AA gel. The sample with higher St content eluted later and one with higher acrylonitrile content eluted earlier. The slope for AA gel is slightly steeper than one for AN gel. A mixture of eight samples with different compositions was separated by two HPLC: first using AN column and then using AA column (Figure 3). As shown in Figure 3(b), the sample was separated into each component. Thus, terpolymer was successfully separated by composition using HPLC cross-fractionation. The RP HPLC using St column showed almost the same slope as AA column, although elution order was opposite to NP HPLC.

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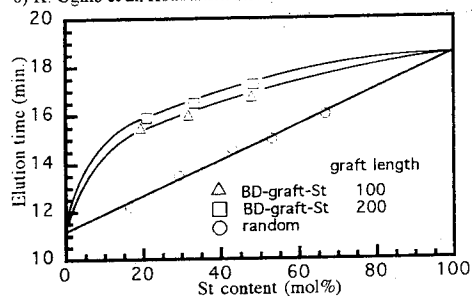
Fig. 1 Relationships between elution time and St content of St-BD copolymers
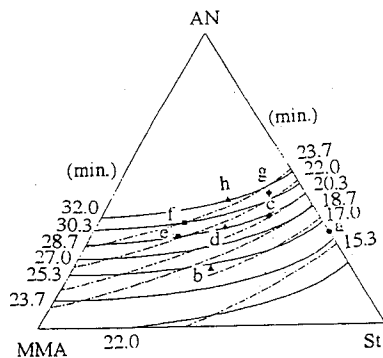
Fig. 2 Elution times of terpolymers
— AN column ···· AA column
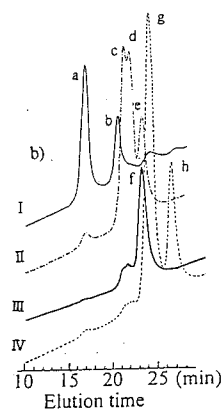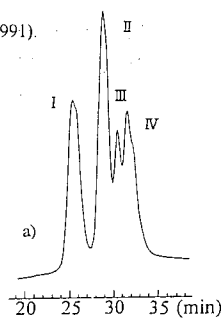
Fig. 3 Crossfractionation of terpolymers
a); AN column, b); AA column

POLYMERS AT INTERFACE AND BIO CHROMATOGRAPHY

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The use of porous solid beads for the separation of natural polymers is limited by the occurence of several kinds of interactions that promote in many cases irreversible adsorption. The coverage of silica by polymers has opened new ways to reduce the type of interaction involved in the separation processes and to cancel the irreversible binding. We present here different studies on the structure of size exclusion, ionic, and affinity supports prepared by coating silica with hydrophilic and reactive polymers.

The interaction of these polymers with silica surfaces have been studied by IR spectroscopy that permits to measure the contacts between silanols groups and polar moities of the macromolecular chains. The preferential adsorption of a part of the segments is thus evidenced. By RPE measurements the segments mobility is also measured that gives a good approach of the chain conformation at solid interface. The role of cross linking obtained by chemical of radiochemical treatments has been analyzed with the different supports. Significative reduction of secondary interactions are revealed with size exclusion supports while some decrease of porous volume is observed. A strong improvement of resolution is obtained by the cross linking of charged polymers used to prepare ionic supports. The role of coating procedure, binding process and cross linking was analyzed by determination of mass transfer kinetic parameters for all supports. Finally the use of polymer immobilized at solid surface is a valuable mean to obtain chromatographic supports. Nevertheless the structure of the layer must be carefully controlled to obtain homogeneous and stable coverage essential for useful supports in biochromatography.

THE DEEP SIGHT INTO THE LIQUID CHROMATOGRAPHY OF POLYMERS UNDER THE CRITICAL CONDITIONS

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In the recent years the method of critical chromatography has received a wide application. In the chromatography under the critical conditions there is no separation of macromolecules according to molecular weight, all the polymeric molecules appearing from the column simultaneously, together with the solvent molecules.

The structure of macromolecules inside pores under the conditions of critical chromatography is considered. An ideal polymer chain in a slit-like pore serves as a model. The geometric and thermodynamic characteristics of a macromolecule are obtained, namely the mean sizes of a polymer molecule, its size fluctuations, end-to-end distance distribution function, in-pore chain unit density profile, mean number of contacts between chain units and pore walls, etc. These parameters are calculated as the functions of the distance between macromolecules and pore walls.

Recent achievements in the theory and applications of the liquid chromatography under the critical conditions are discussed.

COMPOSITE DEXTRAN GELS FOR SIZE EXCLUSION CHROMATOGRAPHY

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.Modified dextran gels crosslinked covalently with epichlorohydrin and additionally by coordination links between polyvalent metal cations and OH groups of the polysaccharide have been prepared and investigated using size exclusion chromatography (SEC).

The composite gels contain respectively the following elements: Mg, Zn, Ca, Mn, Co, Ni, Fe, Al, Ti, Cu, Ag, Si and B.

In dependence on a kind of the modyfing agent: typically a metal salt or oxide, the composite spherical dextran particles were obtained differing in their swellability in aqueous media as well as in SEC characteristics. Poly(ethyleneglycol) standards and water mobile phase were applied in SEC measurements.

The modified dextran gels swell in water to higher or lower degree than the reference dextran/epichlorohydrin gel [1] in dependence on a type of the modyfing agent and its concentration. Fig. 1 presents a case of the composite heterogenous gels with lower swellabilities than the reference gel. Ti containing gels swell in water substantially less than the other investigated materials.
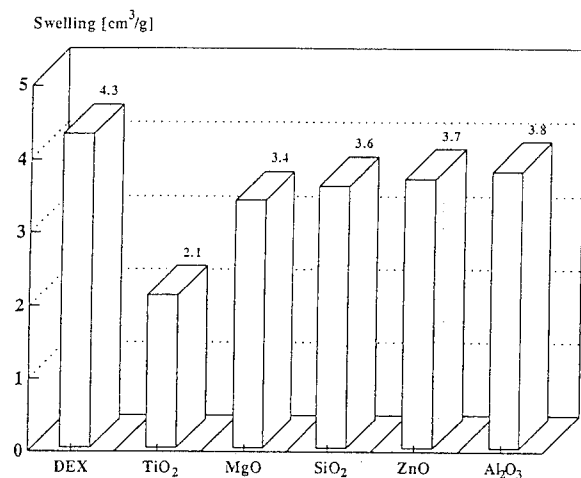
Fig. 1. Water swellability of the dextran gel crosslinked with epichlorohydrin (1:1.5 mol/mol) - DEX, and the dextran composite gels prepared in presence of various oxides (1 g oxide/ 3.0 g of dextran)

The large shifts of the calibration curves measured for PEG standards were found for the gels prepared in presence of the same concentration of the modyfing agent (Fig. 2). Differences in separation characteristics of the hydrogels are discussed. Nonexclusion mechanisms of separation caused by different ability of the cation to coordinate residual functional groups of the dextran and various porous gel structure seems to be main factors influencing the hydrogel properties.
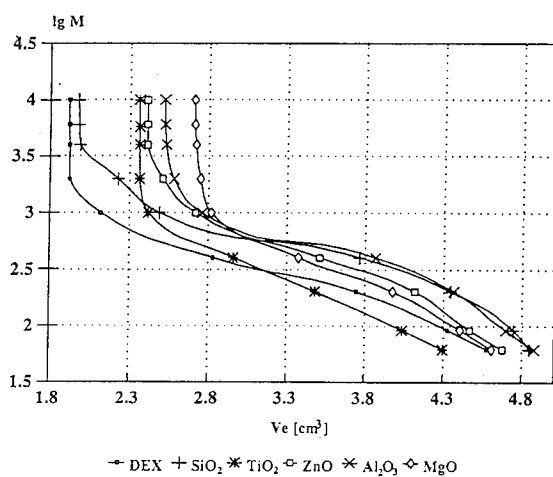
Fig. 2. Calibration dependences of the dextran gel crosslinked with epochlorohydrin (1:1.5 mol/mol) - DEX, and the dextran composite gels. Eluent - water, standards - poly(ethylene glycol)

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MOLDING, A NEW APPROACH TO THE PREPARATION OF SEPARATION MEDIA FOR CHROMATOGRAPHY OF POLYMERS

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Rods of macroporous poly(glycidyl methacrylate-co-ethylene dimethacrylate) and poly(styrene-co-divinylbenzene) have been prepared by a molding process that proceeds directly within the confines of a chromatographic column and their use for HPLC separation of biopolymers and synthetic polymers have been studied.

Porosimetric study revealed a large volume of pores with a diameter of about 1 μm. The presence of these large pores makes the molded rod columns easily permeable to eluents, and therefore the back pressure is modes even at high flow rates. In contrast to the conventional HPLC columns packed with beads, all the mobile phase flows through our continuous medium. Mass transfer between the stationary and mobile phase is very fast as a result of the convection and the efficiency is almost independent of the flow rate. This also improves significantly the separation ability of the rod columns in certain chromatographic modes and very fast separations of macromolecules can be achieved.

Proteins and peptides were separated in a reversed-phase mode using a gradient of acetonitrile in a buffer solution. For example, excellent resolutions of three proteins were achieved even at a flow rate of 25 mL/min and a mixture of 4 proteins was separated within 24 seconds.

High-performance precipitation chromatography of styrene oligomers and polymers was performed using a mobile phase gradient of nonsolvent in a good solvent. Homopolymers were easily separated in a 50 mm long molded column within a few minutes according to their molecular weights. In addition, copolymers can also be separated according to their chemical composition.

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CHEMICAL COMPOSITION DISTRIBUTION OF GRAFT COPOLYMERS PREPARED BY MACROMONOMER TECHNIQUE: DETERMINATION BY GRADIENT HPLC

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In the graft copolymers prepared from macromonomers and small comonomers, the branches are approximately uniform but the backbones are heterogeneous in the degree of polymerization. From this structural feature, very broad chemical composition distributions (CCDs) and other interesting characters of CCDs may result. Important factors controlling CCDs of the graft copolymers may be (a) degree of polymerization (DP), (b) macromonomer content, (c) molecular weight (MW) of macromonomer (graft length), (d) conversion and (e) end-group of macromonomer. However, only a few studies on the determination of CCDs of the graft copolymers have been published.[1-4] The CCD of PMMA-graft-PDMS prepared by the macromonomer technique was determined by demixing-solvent fractionation by Stejskal et al.[1]. However, the method may not be applied generally, since it is very difficult to fined a suitable pair of demixing solvents for a given graft copolymer. On the other hand, we determined the CCDs of PMMA-graft-PS samples using gradient HPLC.[2-4] This method can be applied more readily than the former method for the present purpose.

In the present lecture, I will discuss the features of CCD of the graft copolymer, using our experimental results. For the effect of (a), it is too natural from the theoretical point-of-view that the higher the DP, the sharper is the CCD. In the present research, therefore, the DPs were controlled to be not so different each other to affect on the CCDs seriously, for all samples. The factors from (b) to (e) were examined experimentally.

The graft copolymer samples were prepared by radical copolymerization of PS macromonomers prepared by living polymerization having methacryloyl (MA) and p-vinylbenzyl (VB) end-groups with MMA. The macromonomers of PS(MA) of three different molecular weights were used. The samples of the

respective series were copolymerized from the monomer feed of three different compositions. Different conversion samples were prepared from the same composition monomer-feed. HPLC measurements were carried out by using a cyano-modified silica-gel column and gradient elution of tetrahydrofuran (THF) and n-heptane for the normal-phase (NP) mode, and a octadecyl-modified silica-gel column and gradient elution of THF and acetnitrile for the reversed-phase (RP) mode,respectively. The chromatograms were converted to CCDs of the samples by an optimization method. The theoretical CCDs were calculated using the theory of Stejskal and Kratochvil[5] and shown in the below figure (dashed curves).

As the results: (1) Both CCDs obtained by the NP and the RP modes were in good agreement to one another even for the high-conversion samples with asymmetric CCDs. This indicates that the effect of the molecular weight distribution on the CCD is negligible. (2) It is seen in the below figure that the CCDs are very broad even for low-conversion samples, and that the CCD becomes broader as the macromonomer content decreases, and also that the CCD becomes broader as the graft length increases if comparing samples with similar average composition. These are the features of the CCDs of graft copolymers of this kind and in agreement with the theoretical predictions, as seen in the figure. (3) As the conversion increases, the CCDs of the samples obtained from the same composition monomer feeds become broader towards the higher macromonomer-content side for the samples prepared from the PS(MA) and towards the lower macromonomer-content side for the samples from the PS(VB). These dendencies of the broadening of CCDs and the copolymerization data show the strong effect of the intrinsic reactivity of the polymerizable end-group on the copolymerization reactivity of the macromonomer.

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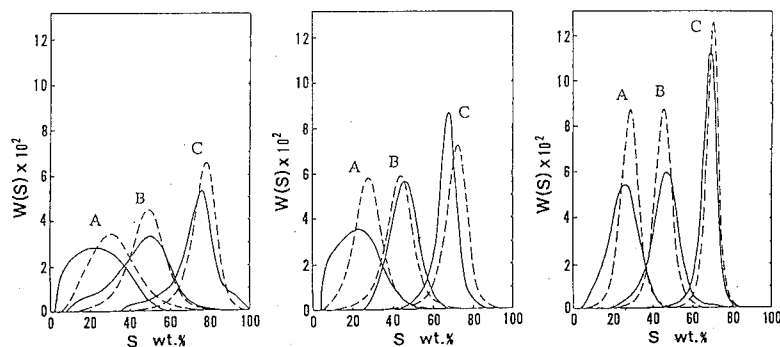
MONITORING OF C5 - C30 ORGANIC COMPOUNDS IN POLYMERS BY SPE, SPME AND THERMAL DESORPTION

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To maintain product quality and to evaluate product content for potentially toxic components, polymer manufacturers routinely need information about the composition of their product.

Thermal desorption at specific temperatures can quickly and reliably produce fingerprints of C5–C30 offgassed components. Monitoring this wide range of compounds requires an adsorbent tube filled with special graphitized carbon black adsorbents.
Several features of the SUPELCO Thermal Desorption Unit are also critical to the success of the procedure. The ballistic heating capability ($30°C \Rightarrow 300\pm1°C$ in 20 ± 2 seconds!) of the unit is essential.

Volatile components can also be analyzed by headspace SPME (Solid Phase MicroExtraction): a specially coated fused silica fiber is introduced into a heated vial containing the sample, and the organic analytes absorb into the phase. The analytes are desorbed from the fiber to a capillary GC column by the heated chromatograph injection port. The fibers can be re-used, no solvents or complicated apparatus are required, and ng/L detection limits can be attained with GC/ITD.

Semivolatile components or polymer additives from real samples are difficult to analyze : time-consuming with many steps such as Soxhlet extraction followed by clean up and preparation for chromatographic analysis. A complete Soxhlet extraction may require 48 hours and large sample size. At the same time, SFE (Supercritical Fluid Extraction) provides better extraction efficiencies due to the low viscosity and high diffusivity of supercritical fluids and produces an order of magnitude increase in the rate of extraction.
Another advantage of SFE is its potential for selective extraction which can be performed by altering the conditions, particularly temperature and density, and so time-consuming clean-up steps can be avoided.
SFE has also been incorporated on-line with various chromatographic techniques such as capillary GC and SFC.

MASS DETECTION IN GEL PERMEATION CHROMATOGRAPHY USING A SINGLE CAPILLARY VISCOMETER

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Gel Permeation Chromatography is a very powerful tool for the characterization of polymers. Generally, mass detectors like viscometric detector or light scattering detector are used to get more information about the polymer. Viscometric detection is particularly useful to use "universal calibration". The coupling of the refractive index detector with viscometric detection allows the determination of branching distribution and the calculation of true average molecular weights using a universal calibration curve.

The single capillary (SCV) design is a direct extrapolation of classical viscometry measurement. It is composed of a small capillary through which solvent flows at constant flow rate and a pressure transducer which measures the pressure drop across the capillary. SCV obeys Poiseuille's law and the pressure drop P across the capillary depends on capillary geometry (radius r and length l), on flow rate Q and on viscosity of fluid eta according to:

$$P = 8 / pi * l / r 4 * eta * Q$$

At constant flow rate Q, the pressure drop is proportional to viscosity eta and at constant viscosity eta, the pressure drop is proportional to flow rate Q. Consequently, in order to use the SCV as an accurate viscometer, the flow rate must be maintained absolutely constant during the GPC experiment. Conversely, SCV allows perfect control of flow rate and can also be used as a very powerful troubleshooting tool.

The reason in using here a differential refractometer DRI prototype (#4) is that it has been demonstrated that a standard DRI detector may lead, in certain conditions, to erroneous results in viscosity calculations because of the occurrence of a very small flow fluctuation so-called "Lesec effect" when the polymer flows across the detectors.

This very small fluctuation is induced by the specific viscosity of the polymer solution and is enough to produce a significant apparent shift downstream of the viscometer peak that leads to an apparent small rotation of the viscosity-molecular weight relationship and an apparent decrease of the Mark-Houwink exponent alpha for linear polymers or a distortion of the viscosity law for branched polymers. This has been previously described. DRI prototype (#4) has been especially designed to reduce this effect. A special geometry is used to reduce the pressure drop in the detector area but also the void volume. This design is used in the new WATERS GPC 150CVplus.

GPC-Viscometry requires a molecular weight calibration curve, usually a "Universal calibration curve" Log ([eta]*M) = f (Ve). With classical GPC, using only one concentration detector, perfect control of solvent flow rate is required since molecular weights of broad polymers are calculated by comparison of

their sliced distributions with elution volumes of narrow standards. Data acquisition being performed as a function of time, this comparison can be achieved accurately only when both experiments are run at exactly the same flow rate. A small error in flow rate introduces a significant error in molecular weight because of the logarithmic scale of the calibration curve. The purpose of this paper is not to discuss this kind of problem, but to study the consequence of very small flow fluctuations. These fluctuations are unable to introduce a significant error in molecular weights when referring to a calibration curve, but they lead to very small peak distortions with flow-sensitive detectors like the Single capillary Viscometer (SCV) and, consequently, to errors in data interpretation.

APPLICATIONS OF MULTIDETECTION IN THE GEL PERMEATION CHROMATOGRAPHY ANALYSIS OF COMPLEX POLYMERS

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Gel Permeation Chromatography is a very powerful tool for the characterization of polymers. For the study of complex polymers with a very complicated structure, the use of multidetection technique greatly increases the power of characterization. Generally, mass detectors like viscometric detector and light scattering detector are used to get more information about the polymer. A WATERS 150CV (refractive index DRI and single capillary viscometric detection) with an on-line light scattering detector (LALLS-CHROMATIX CMX 100) were used for the characterization of star-branched model copolymers. The coupling of the DRI with the viscometric detection allows the determination of branching distribution and the calculation of average molecular weights using a universal calibration curve. Also the coupling of the RI with the light scattering detection provides the average molecular weights.

These polymers were synthesized in Liege University (Belgium). The polymer branches are composed of polymethylmethacrylate (PMMA) / polytert-butylacrylate (PtBA) di-block copolymers with a very well controlled chemical composition and structure (very low polydispersity). These branches were chemically coupled to form star-branched copolymers with a various number of branches, the problem being to determine the number of branches.

The problem is to determine the number of branches of star-branched model copolymers, the number average Mn is the key parameter. The light scattering detection is not the most appropriate method to determine the number average Mn but provides the weight average Mw with a good accuracy. The viscometric detection provides results assuming that the universal calibration is perfectly observed. In this work, we have used the light scattering detection to determine Mw and to compare these values to the ones obtained by the viscometric detection.

The comparison of Mw allows to validate the use of the universal calibration curve and, therefore, the use of the number averages Mn coming from the viscometric detection to characterize the star-branched copolymers.

It has been found an excellent agreement between results coming from viscometric detection and universal calibration and the ones from LALLS, that demonstrates an excellent performance for the universal calibration, even for so branched polymers with a very particular viscometric behavior. Mn values from stars and branches were used to determine the number of branches of the star-branched copolymers. The values obtained with the viscometric detection and the LALLS. The calculation of the number of branches was performed by making the ratio of the number average molecular weight of the star polymer to

that of the uncoupled linear branches. The use of Mn from GPC/Viscometry and Mn from GPC/LALLS leads approximately to the same number of branches in the star-branched copolymers. The ratio [eta]br/[eta]lin, between the intrinsic viscosity of the star and the one of the linear molecule used to synthesize the star, is found to be approximately constant and equal to 2 that has already been observed for other star branched polymers were found very similar.

Several different copolymers with different compositions and physical structures have been studied. The variations of the branching parameter g' with the number of branches seems to indicate that the copolymer structure is more complicated than a star model structure, the exponent of the g/g' relationship being higher than 0.5 and increasing with the number of branches.

CHARACTERIZATION OF POLY(METHYL METHACRYLATE-GRAFT-3-HYDROXYBUTYRATES) BY GPC AND NMR SPECTROSCOPY

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Validity of the universal calibration principle for characterizing branched polymers by GPC has been questionated, especially with comblike polymers [1].

Therefore, the number-average molecular weights of the new developed graft polymers, obtained via an anionic grafting reaction of β-butyrolactone (β-BL) on poly(methyl metacrylate) (PMMA)[2]:
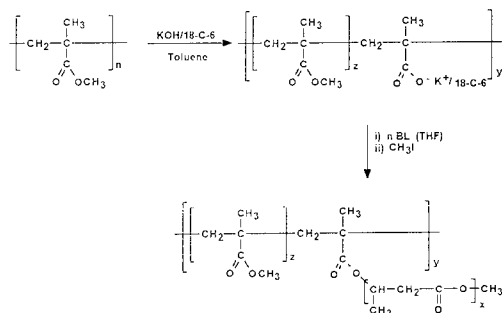
have been estimated according to the formula:

$$Mn = y^*(Mn_z + Mn_x + MA)$$

in which Mn_x is the number-average molecular weight of poly(3-hydroxybutyrate) chains, Mn_z is the number average molecular weight of PMMA backbone consisting of z units and MA is the molecular weight of a methacrylate unit. The average number of poly(3-hyroxybutyrate) units in the grafted chains (x) was estimated from ^{1}H NMR spectra of graft polymers obtained. The average number of the carboxylate initiation sites (y), as well as the average number of the MMA units between the saponificated groups (z), were determined by ^{1}H NMR and GPC measurements of the copolymer obtained after benzylation of the sample of macroinitiator with benzyl bromide [2].

It turned out that estimated Mn values were in good agreement with those obtained by GPC measurements of the graft polymers studied, most probably due to the fact that the number-average molecular weight of β-BL branches is relatively low as compared with that of PMMA.

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SEPARATION POLY (METHYL METHACRYLATE)S WITH DIFFERENT TACTICITY BY MEANS OF LIQUID CHROMATOGRAPHY UNDER CRITICAL CONDITIONS OF ADSORPTION

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Polymers forming stereoregular structures exhibit at least two distributions viz. that of molar mass and that of stereoregularity. It is impossible to assess both distributions by a liquid chromatographic (LC) method which utilizes just one separation mechanism e.g. the size exclusion chromatography (SEC). Moreover, the macromolecules of the same molar mass but of different degree of stereoregularity usually have similar sizes in good solvents.

Recently [1] we have shown that it is possible to discriminate polymers with narrow molar mass distribution differing in their degree of stereoregularity by combining entropic SEC mechanism of separation with the processes dependent on the differences in the solubility of particular stereoisomers (liquid chromatography under limiting conditions of solubility [2]). Poly(methyl methacrylate)s (PMMAs) were chosen as model polymers because they can be prepared with narrow molar mass distribution and high degree of stereoregularity [3] while the solubility of syndiotactic and isotactic polymers differs sufficiently.

In present work we have checked the potentiality of the liquid chromatography under critical conditions of adsorption (LCCC) for such discriminations.

LCCC presents a combination of size exclusion separation mechanism with adsorption separation mechanism. The set of "critical" chromatographic conditions is identified for a given homopolymer (column packing, eluent strength, temperature, pressure) under which the two above counteractive mechanisms mutually eliminate so that no separation according to the molar mass takes place and all macromolecules elute from the column approximately in the total column volume regardess of their molar mass. This behaviour can be utilized in the separation and analysis of interpolymers (block-or graft-copolymers), functional oligomers and polymer mixtures which constituents exhibit different adsorption characteristics: The experimental conditions are chosen usually in such a way that one constituent is set at its critical conditions while another constituent is normally separated in the SEC mode.

We have investigated the LCCC/SEC behaviour of the highly isotactic and highly syndiotactic PMMAs at 30 °C and have found that
- the adsorption differences of homopolymers with different tacticities are large enough to apply the LCCC principle to their discrimination

- the critical conditions can be found either for syndiotactic PMMA (tetrahydrofuran (THF)/n-hexane = 83.2/16.8 % wt/wt) or for isotactic PMMA (either THF/chloroform (CHCl₃) = 12.2/87.8% wt/wt or CHCl₃/ethanol =98/2% wt/wt) so that another polymer is eluted in the SEC mode.
- LCCC separates iso- and syn-PMMAs of similar molar masses if the noncomplexing eluent (CHCl₃ mixtures) is used.

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CHITOSAN PROCESSING IN GPC REPRESENTATION

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Chitin is widespread and reproducible natural linear polymer. Its molecular chain consists of 2-acetylamino-2-deoxy-β-D-glucose units. The main source of chitin are some marine animal organisms, such as shrimps, crabs, krill and others. Utilization of unprocessed chitin for industrial purposes is, however, limited because of its insolubility. Only some derivatives of chitin obtained as the products of its processing are soluble. Among them the most important is chitosan, i.e. partially deacetylated chitin.

Commercially available are chitosans having degree of deacetylation in the order of 70 %. In these polymers about 70 % of the initial acetylaminogroups of chitin is converted into aminogroups. Such polymers are easily soluble in the diluted water solution of organic acids.

In spite of its relatively high price chitosan has found many important applications, e.g. in textile industry, agriculture and lately for biomedical purposes. Particularly valuable properties of chitosan are obtained after its special physico-chemical processing elaborated in the Institute of Chemical Fibres. The product obtained can be better degraded by blood and lymph of mammals. It also reveals excellent sorption properties and is of significant importance for the application in medicine, e.g. as an active component of wound-dressings.

As biodegradability of chitosan plays such an important role in its application, detailed studies on this problem have also been carried out in the Institute. The investigation of chitosan biodegradability is performed with the use of selected enzymes or mixed microorganisms taken from their natural environment.

All the above mentioned processes are examined and controlled by means of gel permeation chromatography (GPC). In the presented communication changes of molecular characteristics, accompanying chitosan processing and its degradation are shown.

For the measurements have been used Hewlett-Packard HP 1050 chromatograph, RI detector HP 1047/A, PL - GFC 4000 A, 8 μm and PL - GFC 300 A 8μm columns, GPC Software Version 4.0 Polymer Lab. Ltd. Continuous phase: 0.33 M CH_3COOH + 0.2 M CH_3COONa, flow rate 1.0 ml/min., temperature of column 25°C.

CAPABILITY OF GRAVITATIONAL FIELD-FLOW FRACTIONATION FOR MICROPREPARATION OF STEM CELLS

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In Gravitational Field-Flow Fractionation (GFFF), similarly as in other techniques of Field-Flow Fractionation (FFF), the separation is reached by a simultaneous activity of an external adjusted force field acting perpendicularly to the direction of a carrier liquid flow. Positions of sample components in the parabolic flow velocity profile of the carrier liquid result from the specific sample properties that are pronounced by the applied force field: these two factors determine the elution times.

GFFF is a suitable technique for separation/characterization of biological samples because experimental conditions can be chosen to be gentle enough to maintain integrity and function of biological structures. The cells can be used for further biological investigation. Another advantage of this method is its simplicity: GFFF utilizes Earths gravity as the external force field, which causes settlement of particles toward the channel bottom. Other forces acting on particles in the carrier liquid flow, namely the hydrodynamic lift-forces, tend to drive particles away from the channel bottom. Thus, the particles are finally focused into narrow zones. GFFF has been already used, e.g., for characterization of blood cells.

This paper describes a study of stem cells from mouse bone marrow. Hemopoietic tissue forms main part of bone marrow. In the procedure used, the hemopoietic cell populations of adult mice are destroyed by a supralethal irradiation dose. The mice are then injected intravenously with small numbers of bone marrow cells and 7-14 days later the spleen is examined for the presence of discrete, macroscopically visible surface colonies. The aim of the paper were to obtain a fraction with stem cells, which can be used for further biological application. Fractions were analyzed by Coulter Counter and applied into irradiated mice.

STUDY ON DEGRADATION OF NITROCELLULOSE BY SEC

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The changes in molecular weight of blasting explosive grade nitrocellulose (NC) during the stabilization stages of the industrial nitration process have been investigated by means of *Size Exclusion Chromatography* in tetrahydrofuran at 36 ^{0} C with simultaneous refractometric (DRI) and spectrophotometric (UV at 254 nm) detection.

The optimized operating conditions for *SEC* analysis were as follows:
- Columns   2 linear PL-gel Mixed-C, 5 μm
- Sample conc.   0.12 % w/v using air dried NC
- Sample solution   allowed to stand overnight, filtered through sintered porous glass
  preparation
- Injection volume   20 μl
- Flow rate   1 ml/min
- DR sensitivity 8×10^{-6} RIU
- UV sensitivity   0.02 AUFS

The molecular weight distributions MWD of NC samples were determined using the conventional calibration with narrow range polystyrenes, followed by conversion to molecular weight by means of the universal calibration principle. The following Mark-Houwink relationships for NC and PS, respectively, were applied:

$$[\eta] = 0.25 \times 10^{-2} \ M,$$

$$[\eta] = 1.17 \times 10^{-2} \ M^{0.717}$$

The influence of (1) boiling time and (2) addition of hexamethylenetetramine during the stabilization stage on MWD of NC was studied.

It was found that the stabilization stages lead to a very large change in M_W. The progressive decrease in M_W with boling time was observed, which corresponded with a general shift of the whole *SEC* envelope to lower molecular weights. This tendency was also noticed at the constant boiling time while varying the concentration of hexamethylenetetramine added.

The possibility of controlled degradation of NC was stated.

MOLECULAR CHARACTERISTICS OF CELLULOSE IN COURSE OF ITS ENZYMATIC CONVERSION

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Cellulose - the most widespread natural polymer has been used for many years as a material in the manufacture of fibres, paper and other products.

All the methods of manufacturing fibres of regenerated cellulose or products of its modification are aimed at improving cellulose solubility in many different ways.

In the Institute of Chemical Fibres Łódź, Poland a new method of obtaining soluble cellulose has been elaborated. The method is based on the ability of cellulolytic enzymes to convert cellulose. The process of enzymatic transformation of cellulose is a new field of research in biotechnology. In the above mentioned process the partial depolymerization of cellulose takes place accompanied by other changes of its physico-chemical characteristics.

The process leading to obtaining a soluble product from which a spinning solution can be prepared causes the change of average molecular weight and also changes the molecular weight distribution (MWD) of the polymer under investigation.

The detailed analysis of the molecular structure of biotransformed cellulose performed by means of gel permeation chromatography (GPC) is important in evaluating the efficiency of cellulolytic enzymes action and in determining the usability the product obtained for the further application in the production of cellulose fibres and films.

Using GPC method the average molecular weight and MWD of cellulose in course of its enzymatic conversion were determined.

For the measurements have been used Hewlett - Packard HP 1050 chromatograph, RI detector HP 1047/A, PL - gel Mixed B, $10\mu m$ column, GPC Software Version 4.0 Polymer. Lab. Ltd. Continuous phase: DMAc/0,5 % LiCl, flow rate 0.8 ml/min., temperature of column $80^{\circ}C$.

RETENTION BEHAVIOR OF OLIGOMER SURFACTANTS ON POLYETHYLENE COATED ZIRCONIA HPLC COLUMN

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The majority of analysis in high-performance liquid chromatography (HPLC) is carried out in reversed-phase (RP) separation mode, therefore many reversed-phase chromatographic supports have been developed with various hydrophobic ligands covalently bonded to the surface of inorganic (silica or alumina) or organic supports. The retention of solutes in RP-HPLC considerably depends both on the physicochemical parameters of the support and those solutes, solute hydrophobicity being the most relevant parameter. However, the acidic or basic groups of supports, not covered by the hydrophobic ligand may influence the retention of polar solutes resulting in retention order different from that predicted according to the molecular lipophilicity. The influence of free silanol groups on the retention was defined as "silanophil effect", it decreases with the increasing density of covalently bonded alkyl chains, and it is generally low or absent on polymer (i.e. polystyrene) coated silica. Not only polystyrene but also other polymers such as octadecyl polyvinylalcohol copolymers, polytrifluorostyrene and polyethylene have been tested as coating agents of silica for the preparation of reversed-phase support. These polymer coated supports show enhanced mechanical and pH stability.

The objectives of the work were the determination of the retention behaviour of some nonionic surfactants on polyethylene-coated zirconia RP-HPLC support and to find the similarities and dissimilarities between the retention characteristics of solutes.

The HPLC equipment consisted of a Gilson gradient analytical system (GILSON Medical Electronics, Villiers-le-Bell, France) with 2 piston pumps (Model 302), Detector (Model 116), Rheodyne injector with 20 μl sample loop (Cotita, California, USA), and a Waters 740 integrator (Milford, Massachusetts, USA). The flow-rate was 1.0 ml/min and the detection wavelength was 235 nm. Eluents were the mixture of methanol and water the methanol concentration varying between 50 – 75 vol.% in steps of 5 vol.%. The polyethylene-coated zirconia support was prepared in our laboratory. Columns of 15 cm x 4 mm I.D. were used in each experiment. The surfactants were ethoxylated nonylphenol derivatives containing in average 4, 5, 6, 8, 9, 10, 11, 13, 15, 23 and 30 ethyleneoxide groups per molecule (Hoechst AG, Frankfurt, Germany). They were dissolved in the eluent at a concentration of 0.05 mg ml^{-1}. The retention time of each compound was determined by three consecutive determinations. The capacity factor and the coefficient of variation of capacity factor were calculated for each compound. As the correlation between the log k' value and the organic phase concentration is generally linear in HPLC we also applied linear equations to

describe the dependence of log k' value on the organic mobile phase concentration:

$$\log k' = \log k'_0 + b.C \qquad (1)$$

where: $\log k'$ = logarithm of capacity factor; $\log k'_0$ = logarithm of capacity factor extrapolated to zero concentration of organic component in mobile phase (intercept, related to molecular lipophilicity or retention capacity of solutes); b = change of log k' value caused by unit change (1 vol %) of organic mobile phase concentration (slope, related to the specific hydrophobic surface area in contact with support), and C = methanol concentration in the eluent (vol %). To test the validity of the hypothesis that in the case of homologous series of solutes the intercept (lipophilicity) and slope (specific hydrophobic surface area) values are intercorrelated linear correlation was calculated between the corresponding parameters of Eqn. 1. In order to elucidate the impact of the number of ethylene oxide group (n_c) on the retention linear correlations were calculated between the parameters of equation 1 and the n_c value.

Each surfactant showed regular retention behavior on the polyethylene-coated zirconia column the logarithm of the capacity factor linearly decreased with increasing concentration of methanol in the eluent. The relationship was significant in each instance. Significant linear correlation was found between the slope and intercept values of Eq.1 indicating that from chromatographic poind of view the surfactants behave as a homologous series of solutes:

$$\log k'_0 = 75.61 + (5.30\pm1.04).b \qquad (2)$$
$$n = 10 \qquad r_{calc.} = 0.8747$$

The parameters of Eq.1 significantly depended on the length of the ethylene oxide chain:

$$\log k'_0 = 89.00 - (0.26\pm0.11).n_c \qquad (3)$$
$$n = 10 \qquad r_{calc.} = 0.6501$$

$$b = 2.57 - (0.056\pm0.015).n_c \qquad (4)$$
$$n = 10 \qquad r_{calc.} = 0.7988$$

The results of eqs 3 and 4 suggest that polyethylene-coated zirconia is a promising tool for the analysis of ethoxylated nonionic surfactants.

CHARACTERISTICS OF PDMA - URETHANE PREPOLYMERS (PSU) SOLUTIONS BY HPLC-GPC, LS AND QELS

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PSU samples were synthesized from tetrametyl-xylilenediizocyanate (TMXDI) and siloxane oligomers.

The PSU samples were investigated using HPLC-GPC (for THF solutions), LS and QELS (for toluene solutions).

Experiments were performed by using:
Shimadzu C-R4A Chromatopac Gel Permeation Chromatograph equipped with two detectors - differential refractometer and UV spectophotometer. Columns were calibrated with polystyrene standards.
LS studies were made using FICA photogoniodiffusiometer and classical Zimm method of double extrapolation. Microgel - containing samples were evaluated according to the Lange procedure.
The QELS measurements were carried out by using the Malvern 7032 N/72C Multi - 8 bit correlator with 72 channels. The light source was a 5 mW He-Ne laser. The time correlation measurements were performed at 90° angle.

The experimental results of this study led to characterization of the process of microgel formation in the dilute solutions of the PSU samples as a function of time.

QUANTITATION OF POLYOXYETHYLENIC SURFACTANTS IN HPLC USING ELECTROCHEMICAL DETECTION

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Objective : We previously described the analysis of polyoxyethylenic surfactants using either reversed phase HPLC (1) or ion exchange/ complexation chromatography (2). We also used polarographic detection of these surfactants derivatized by 3,5-dinitrobenzoyl chloride (3). We report here the quantitative analysis of these surfactants, using the same techniques.

Content : The samples analyzed were obtained from mixtures of C_{12} to C_{18} saturated fatty alcohols with an average number of ethylene oxide (E.O.) from 6 to 25 E.O.. They were esterified by 3,5-dinitrobenzoyl chloride (DNBC) in order to allow polarographic detection (in reduction mode, mercury electrode).

Chromatographic analyses were performed in the following conditions :
- reversed phase analyses were done using a C_8 stationary phase and a water-acetonitrile (2:3) mobile phase containing tetrabutylammonium perchlorate ;
- ion exchange/complexation chromatography was performed using silica or Nucleosil-SAX cation exchanger as stationary phases. The mobile phase was water-acetonitrile (1:9) containing sodium acetate ;

Pure standards (monodispersed $C_{16}EO_6$ and $C_{18}EO_6$ esterified by DNBC) were used to establish calibration curves. A correct estimation of surfactants concentrations and of E.O. distribution was possible up to 50 ppm in aqueous solutions and up to 500 ppm in UV absorbing media, such as a crude oil (some of the surfactants analyzed were used in enhanced oil recovery).

Reversed phase and ion exchange chromatographies gave complementary results : reversed phase chromatography allowed a good resolution of the less condensed ethoxylates. The most condensed ethoxylates were better resolved in ion exchange chromatography up to 40 oligomers. Both chromatographic techniques gaved acceptable quantitative measurements (coefficient of variation : 5 to 10%, precision of 5 to 10%).

Conclusion : The combination of reversed phase or ion exchange chromatography with polarographic detection allowed a correct quantitation of polyoxyethylenic surfactants up to 50 ppm in aqueous solution and a selective and quantitative detection in crude oil, up to 500 ppm. It appears as an interesting quantitative method to analyze surfactants at low concentrations, particularly in non electroactive UV absorbing media.

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GPC SINGLE CAPILLARY VISCOMETRIC DETECTOR - INGENIOUS ACHIEVEMENT OR MISTAKEN IDEA ?

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SUMMARY

The theoretical backgrounds for application of a GPC viscometric (VISC) detector is briefly reminded, and types of VISC detectors and their applications for the GPC system are described. A special attention is paid to the single capillary VISC detector that is applied for the Millipore-Waters GPCV system (1992).

Experimental

Materials

Polystyrene (PS) and polyethylene (PE) standards of various producers (NIST, Dow, American Polymer Standards) have been measured.

Equipment

The Waters 150CV gel permeation chromatograph with a double detector system has been used, i.e. with RI and a single capillary VISC detectors. The ExpertEase software of Waters has been installed for the data acquisition and calculations of results. dditionally, comparative measurements for the PS standards have been performed using the Shimadzu GPC instrument (at room temperature).

Results and discussion

The average molecular weights Mn, Mw and Mz, polydispersity indices such as Mw/Mn ratio, as well as viscometric parameters such as Mv and constants K and a of the Mark - Houwink equation, have been determined. The results, in general, do not conform to the contemporary quality requirements for measuring instruments in an accreditation laboratory. Examples of viscometric results, i.e. the K and a constants of the Mark - Houwink equation for PS standards are given. The differences between results obtained from the calibration curve and from the measurements of standard sample are not acceptable. Critical literature data concerning the Waters GPCV system with a single capillary detector are also quoted.

Conclusions

An attempt to answer the title question is presented. However, it seems that the Waters GPCV system with a single capillary VISC detector, as it is recently designed, cannot be used for reliable determination of viscometric parameters for polymers. Perhaps some improvements might be possible as it is suggested by Lesec. Nevertheless, detailed inspection and validation of the system is inevitably needed.

HIGH-PERFORMANCE LIQUID COLUMN AND/OR MEMBRANE CHROMATOGRAPHY OF PROTEINS

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Proteins have very narrow elution "windows" and, therefore, they are eluted from the column in agreement with the "all or nothing" principle. Hence, for the HPLC separation of proteins steep gradients and even stepwise elution are suitable. It may be concluded that short columns can be used effectively in gradient HPLC. Nevertheless, this point of view is the most controversial problem in the literature [1,2].

It is the drawback of many works that the part of a column on which the whole chromatographic process is carried out is unknown. This part of the column may be determined if the equation of protein zone migration is solved for the distance passed by the zone [3].

Our work is based on the idea of attainment of the quasi- steady state of the zone during gradient chromatography [2]. The part of the column on which the quasi-steady state is attained was called the "working column part".

There can be three cases depending on the steepness of gradients used. In the first case the length of the column is equal to that where the quasi-steady state is established. In the second case the column is shorter than that required for the achievemeht of the quasi-steady state, and in the third case the chromatographic column is longer than the calculated length.

The optimal case is in which the column length is equal to the calculated value. However, it must be taken into consideration that at this length good resolution is achieved. If the resolution is low one can take a longer column and, accordingly, use a shallower gradient.

It has been determined for steep gradients or low gradient time (1-5 min) only a short part of the column is used for developing of the chromatographic process. The remaining part of the column is a ballast in which the protein zones move in the regime of parallel transfer without following improving resolution.

As a result, for good separation of proteins very short columns or even flat sheets, i.e.membranes, can be used. It has been shown that in many cases High Performance Membrane Chromatography (HPMC) [4] is more appropriate than HPLC. HPMC can be used to separate proteins with good resolution in a short time. The use of steep gradients or stepwise elution decreases the time necessary for separation and the amount of mobile phase used, thus decreasing the cost of separation process. This is particularly important in preparative chromatographic separation.

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2. S.Yamomoto, et.al., Ion-Exchange Chromatography of Proteins, Marcel Dekker, New York and Basel, 1988.
3. B.G.Belenkii, et.al., J.Chromatogr., 645 (1993), 1.
4. T.B.Tennikova, F.Svec, J.Chromatogr., 656 (1993),57.

ON LINE SIZE EXCLUSION CHROMATOGRAPHY - FOURIER TRANSFORM INFRARED SPECTROSCOPY (ftir): INVESTIGATION OF PREFERENTIAL SOLVATION EFFECTS

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The application of solvent mixtures is a special technique in size exclusion chromatography (SEC) to characterize polymeric and oligomeric substances. The composition of the eluent mixture influences the retention behaviour of the compounds. It is possible to investigate the separation process by combination of pure concentration detectors with spectroscopic detectors. This double detection enables additional information about the chemical nature of the eluated species. The separation experiments were carried out by a direct coupling of a SEC with a FTIR-spectrometer via flow cell interface. N-methylsuccinimide was used as low molecular weight model substance. Beside the concentration detection of substances
the chromatographic peaks of substance and water were identified spectroscopically.
In separation experiments with tetrahydrofurane (THF) as eluent with a water content smaller than 2% it was found that water was eluated in connection with compounds containing imide functionalities as polar groups during the SEC separation. At the retention time of water a water vacancy could be detected. It was concluded that the polar groups were solvated by additional water molecules of the eluent. Such effects of preferential solvatation however in polymer solutions were investigated by light scattering measurements of high molecular weight model substances.

INVERSION OF COMPONENT EXIT ORDER IN FRONTAL CHROMATOGRAPHY OF BIOPOLYMERS

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The analysis of kinetics and dynamics of sorption, which were suggested by Samsonov and Elkin (Sorption & Chromatography of Organic Ions. In "Ion Exchange & Solvent Extraction", Ed. by J. Marinsky N.Y.: Marcel Dekker, Inc. 1984, p. 211) makes it possible to predict the conditions of the sorption regimes realization which the most favourable for separation of mixtures of substances such as polypeptides, proteins, polysaccharides, lipopolysaccharides or their macromolecular complexes. In real systems we have dealt with mixtures of such biologically active substances, where the proportions between the concentration of final products (FP) and contaminants (C) vary in wide limits. For isolation of such macromolecular labile substances and for their separation from C of similar nature it is more useful with help of by varying sorption process parameters to reach the inversion of component exit order than to concentrate the contaminated FP on sorbent. This gives the possibility to purify the FP in a simple frontal process. In the case of proteins it is possible to use for example the changing of one sorbent or carrier in another but with the different functional groups and the same matrix. This gives the strong changing of distribution coefficient Kd of FP or C. In one case in breakthrough solution there are C but FP remain on sorbent. In the other case there are FP in breakthrough solution, but C remain on sorbent. The theory of sorption dynamics predicts the possibility of transition from sorption chromatography to sieveing one, when we retain the quasiequilibrium (regular) condition. In this case we can see the inversion of component exit order for substances, which have different equilibrium and kinetic characteristics. It is important, that the separation may be achieved not only in the conventionally applied elutive chromatographic process, but in frontal one which is favourable for the preparative purification. It is demostrated in Fig. 1 and Fig. 2 by the example of systems including the enzym of hyaluronidase or lipopolysaccharide as FP and proteins as C. In Fig. 1 and Fig. 2 the values of Co are initial concentrations of components in solutions gouing into column, Ci is concentrations of components in the filtrate volume, Ve, after columns.
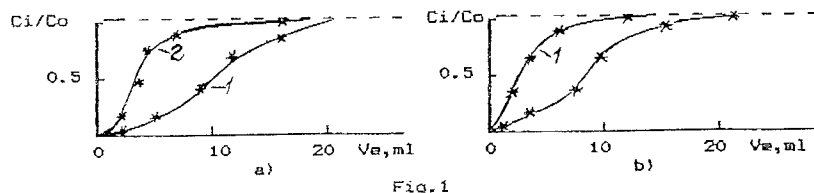
Fig. 1

Fig. 1. shows the breakthrough curves of hyaluronidase (1) and C (2) sorption from their mixture on sorbents which have the same macroporous styrenedivynilbenzene matrix. In the first case on the matrix there are sulfonated groups, in the second case the matrix was modified with cation surface active substance. As we see from Fig. 1 the changing of functional groups led to inversion of component exit order.

Analysis of sorption kinetics and dynamic of slow diffusing substances shows that an efficient separation is possible under irregular regime as well, i.e. under the condition far from equilibrium state. It is conditioned by the fact that the separation ability under such a regime is determined not by the difference in equilibrium coefficients of distribution between the sorbent and solution phases, Kd, but the difference in products Kd D, where D is an intraparticle diffusion coefficient.

Fig. 2. (a, b, c) shows the breakthrough curves of lipopolysaccharide (1) and C (2) in sorption processes on macroporous copolymer modified with cation surface active substance. There are three cases of exit order from column: a) when the frontal sorption process takes place with low flow rate (w= 1×10^{-4} m/s, this is the regular sorption regime) the front of C is the first going out column,

b) when the flow rate is increased to w=6.3×10^{-4} m/s (this is transition to irgular regime) C go out column as the second front,

c) when w is increased to 10×10^{-4} m/s (this is some more far from equilibrium state) both C and L go out column together with solvent.

Fig.2.

- 77 -
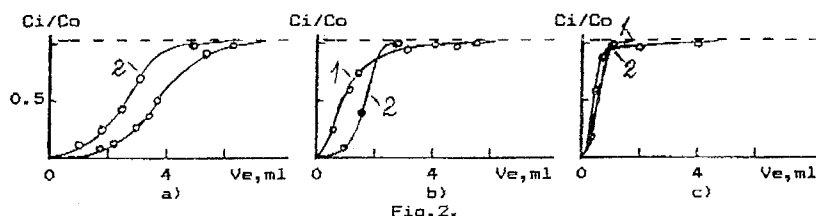
USE OF ALUMINA SUPPORT FOR THE SEPARATION OF ETHOXYLATED OLIGOMER SURFACTANTS ACCORDING TO THE LENGTH OF THE ETHYLENEOXIDE CHAIN

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Many high-performance liquid chromatographic (HPLC) methods have been developed for the separation and quantitative determination of nonionic surfactants. As the separation power of ethylene oxide oligomer surfactants on common reversed-phase HPLC columns is relatively low, diol columns hve also been used for the separation. Nonionic surfactants are generally separated on one HPLC column either according to the length of the ehtyleneoxide chain or according to the typ of the hydrophobic moiety (reversed-phase separation mode). To carry out separations in both senses, two different HPLC columns are needed. Owing to its higher isoelectric point and higher stability in the alkaline pH range, alumina partially or totally overcomes the difficulties arising from the low stability of silica, and therefore its application as a stationary phase for adsorption or after modification for reversed-phase HPLC offers considerable advantages. The objectives of this work were to study the retention behavior of nonylphenol ethylene oxide oligomer surfactants on an alumina column, and to elucidate the effects of various eluent mixtures on the retention.

Alumina column of 25 cm x 4 mm I.D. was used in each experiment. The HPLC equipment consisted of a Gilson gradient analytical system (GILSON Medical Electronics, Villiers-le-Bell, France) with 2 piston pumps (Model 302), Detector (Model 116), Rheodyne injector with 20 μl sample loop (Cotita, California, USA), and a Waters 740 integrator (Milford, Massachusetts, USA). The flow-rate was 1.0 ml/min and the detection wavelength was 280 nm a wavelength where each surfactants show adequate absorption. Eluents were the mixtures of n-hexane - dichloromethane, n-hexane - dioxane, n-hexane - tetrahydrofuran, n-hexane - chloroform and n-hexane - ethyl acetate. The surfactant was the mixture of ethoxylated nonylphenol derivatives containing in average 4 ethyleneoxide groups per molecule (Hoechst AG, Frankfurt, Germany). It was dissolved in the eluents at a concentration of 0.05 mg ml^{-1}. The retention time of each fraction was determined by three consecutive determinations. The capacity factor and the coefficient of variation of capacity factor, the theoretical plate number and the assymetry factor were calculated for each fraction in each eluent system. Principal component analysis followed by two dimensional nonlinear mappind and cluster analysis were used for the elucidation of the similarities and dissimilarities between the elution characteristics of the various eluent systems.

The sample contained four different fractions. It was assumed that each fraction corresponds to an oligomer with a defined number of ethylene oxide group. As we cannot find pure standards to identify the fractions we supposed that the first fraction containd one, the second fraction two

ethylene oxide groups per molecule, etc. The data indicate
that the number of ethylene oxide groups governs the reten-
tion of oligomer surfactants. The shoulders on the chromato-
grams indicate that the fractions contain surfactnats with
different hydrophobic moiety (the apolar alkyl chain is
longer or shorter with one unit), however, these fractions
are not well separated on the alumina column. The theoreti-
cal plate number of the column was the highest for n-hexane
- chloroform eluents followed by n-hexane - ethyl acetate,
n-hexane - tetrahydrofuran, n-hexane - dichloromethane and
n-hexane - dioxane. These data indicate that the best sepa-
ration can be achived by using chloroform as eluent modifi-
cator. Similarly to the theoretical plate number the assy-
metry factor of the fraction was approximately one in n-
hexane - chloroform eluent and it was lower in the other
eluent systems. Principal component analysis proved that the
eluents containing ethyl acetate and chloroform are similar
to each other and the eluent containing terahydrofuran shows
the highest deviation from the other eluent systems. The re-
sults of the cluster dendogram entirely supported the con-
clusions drawn from PCA indicating the both methods can be
successfully used for the evaluation of the characteristics
of various eluent systems on alumina HPLC column.

CHROMATOGRAPHIC PURIFICATION OF ENZYME MEDICINES FROM MACROMOLECULAR IMPURITIES

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The use of sorption and chromatographic methods allows to realize the effective isolation and purification even of such labile substances as enzymes (E). It is particularly important when isolation of E takes place from great complicated systems for instance from extracts of the organs of animals. They often contain some protein and/or polysaccharide contaminations (C) besides basic components. (See Fig. 1 - The gelchromatogram of the bovine testes extract). The methods of fractional precipitation with organic solvent often don't realize complete removal of C which deteriorate the quality of medicines for injections. For improving the quality of such medicines the sorption methods with the use of ion exchangers and/or the molecular sorbents are most perspective particularly for the isolation of pancreas E [1]. For the removal of C from the solutions of hyaluronidase (HY) and ribonuclease (RNA-ase) at different stages of injection medicine production the polymerization sorbents have been used. So for isolation and purification of HY from the bovine testes extracts the sulfonated and nonsulfonated macroporous copolymers of styrene and divinylbenzene have been used. The HY has been concentrated on macroporous sulfonated cation exchanger in quasi-equilibrium regime of sorption. The optimization of sorption and desorption conditions permitted to concentrate E in 5-6 times. More fine purification of HY from eluate has been realized on molecular copolymer of styrene and divinylbenzene. Separation of components was reached by varying the flow rate, W, through the sorption column under the transition from regular (equilibrium) regime of sorption for protein C but not for HY ($1/W=7.5 \ 10^2$ s/m) to irregular (non equilibrium) one ($1/W=2 \ 10^3$ s/m) [2]. (See Fig. 2 -The plot of the retention volumes of HY and protein C differences versus 1/W). As we can see from Fig. 2 there is the optimal flow rate which gives the best separation of HY and protein C. Besides the purification of extracts from macroquantities of protein C it is particularly important to remove the microquantities of C (i.e. purogenic C) which deteriorate the quality of medicines. It was shown [3] that bacterial endotoxins (BE) are wide-spread pyrogenic C in RNA-ase medicine. They are high molecular lipopolysaccharides - cell-wall components of the Gram-negative bacteria. Sorption of BE from the pyrogenic RNA-ase medicine has been realized on the copolymers of dimethacrylatethylenglycol (DMA) and glycidilmethacrylate (GM-label) with different quantities of cross-agent, DMA, (G-label) and styrene (GS-label). These materials were synthesized jointly by the Institute of Macromolecular Chemistry Chem. Acad. N. and the High Molecular Compounds Institute of RAN. Sorption of BE was realized in static as wellas in dynamic conditions. On the Fig. 3 - the gelchromatogram of RNA-ase

medicine solutions is shown before (a) and after (b) the sorption of BE. The contents of BE was controlled by three methods:
1) by asymmetry of gelchromatographic peak, B/A [4];
2) by area of polysaccharide peak, S1 [5];
3) by LAL-test [3].

Fig.1

Fig.2
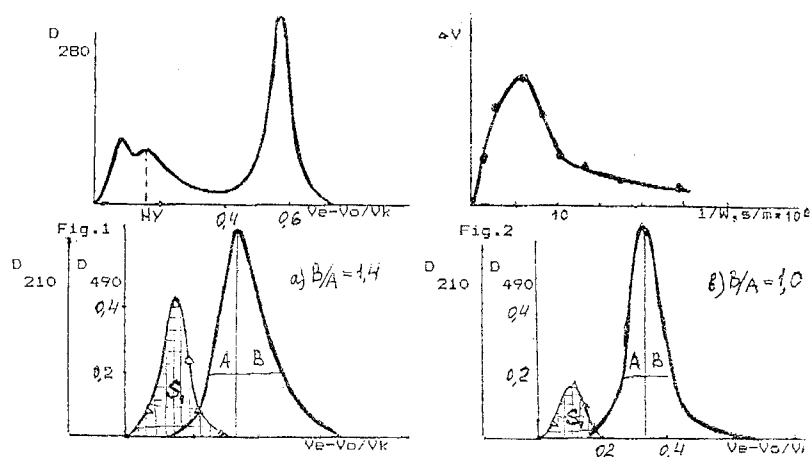
Fig.3

Literature

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[2] Samsonov, G.V., Elkin, G.E., Equilibrium and nonequilibrium dynamics of sorption and chromatography on ion exchangers with polyelectrolute surface layer. J. Polym. Sci., Polym. Symp. 68 (1980) 157
[3] Sitnicov, A.G., Glazova, N.V. et al., LAL-test. The modern approaches to determination of pyrogeneity. St. Petersburg: Tech-Biom, (1993) 107
[4] Glazova, N.V., Dmitrenko, L.V. et al.,/Chem.-Pharm. J., 12 (1991) 79
[5] Glazova, N.V., Ephimova, T.B., Dmitrenko, L.V., J. Phys. Chem. (Rus) 68 (1994) 1803

MOLECULAR CHARACTERISTICS OF GLUCANS BEFORE/AFTER ENZYMATICALLY CATALYZED ACTION OF PURIFIED α (1→4)-GLUCAN-6-GLYCOSYLTRANSFERASE

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An initially α(1→6) long-chain branched α(1→4) glucan was modified by an purified α(1→4)-glucan-6-glycosyltransferase. This enzyme is supposed to produce high molecular short-chain branched glucans from non-branched and/or long-chain branched glucans by a dual mechanism: generation of glucan-fragments of short-chain dp-type by hydrolization of α(1→4) glycosidic linkages, and re-fixing of fragments with α(1→6) glycosidic linkages.
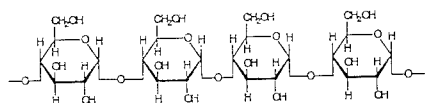
α(1→4)-glucan-6-glycosyltransferase in aqueous solution
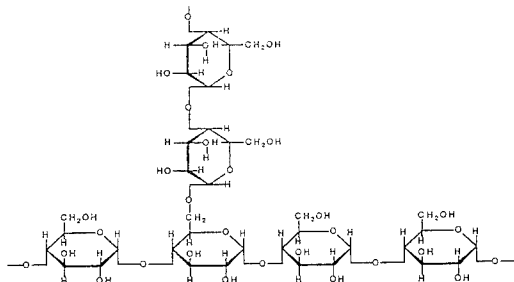
Improved understanding of this enzymatically catalyzed action on the one hand provides a tool for future selective synthesis of glucans with specified branching characteristics and, on the other hand, represents a chance to establish/test correlations between molecular characteristics and macroscopic material properties in a controlled way.

The 'before and after transferase action' glucan-characteristics were investigated by means of size-exclusion chromatography combined with simultaneous detection of mass and and scattering intensity. Additionally, universal calibration was applied to get access on polymer-coil dimensions.

TREF - A POSSIBLE WAY OF STRUCTURAL INHOMOGENEITIES DETERMINATION IN PE COPOLYMERS

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Abstract

The possibility of determination of SCB distribution, and also some other structural peculiarities in copolymers by combination of Temperature Rising Elution Fractionation method and High Temperature Gel Permeation Chromatography is briefly discussed. As an example the analysis of commercial copolymers of ethylene with 1-butene is presented.

LIMITING CONDITIONS IN THE LIQUID CHROMATOGRAPHY OF POLYMERS

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Limiting conditions of solubility are accomplished by employing a mobile phase which is a poor solvent, or even a non-solvent, for the polymer probe. Thus far these have been binary mixtures which, at the temperatures used for the measurement, consists of a thermodynamically good solvent and a non-solvent. The polymer is, however, injected in a thermodynamically good solvent. In this case, homopolymers with different molecular weights elute from the chromatographic column at the same retention volume which is roughly equal to the volume of liquid in column. That is, under limiting conditions there is no separation according to molar mass, as is also the case for critical conditions which are based on a different premise. This permits the method to be applied to separations based on other properties of a given polymer, or for polymer mixtures, as will be discussed.

The following mechanism is believed to cause the limiting condition phenomena: at low levels of non-solvent, such as methanol in toluene or water in tetrahydrofuran for the polystyrene or polymethylmethacrylate systems, the calibration curves shift slightly to lower retention volumes due to the influence of adsorption, partition and a reduced pore size. At higher quantities of non-solvent, in the vicinity of the theta-composition (e.g. 79.6% of toluene for polystyrene in toluene-methanol at 25 °C) the thermodynamic quality of the solvent is strongly reduced. Mixtures containing more methanol are non-solvents for polystyrenes. If such a mixture is used as an SEC eluent and the polymer is dissolved in a good solvent (e.g. toluene), the macromolecules move together with the zone of their initial solvent. If macromolecules move faster due to exclusion processes they encounter the non-solvent and precipitate. They then redissolve as the injection zone (pure solvent) reaches the precipitated polymer. This 'microgradient' process of precipitation-redissolution occurs many times throughout the column with the polymer eluting just in the front part of the solvent zone. As a consequence, the macromolecules move with a velocity similar to the velocity of the solvent zone and elute at the 'limit' of their solubility, hence the nomenclature 'limiting conditions'. Because the polymer elutes very close to the solvent peak, mass specific detectors such as those based on evaporative light scattering, are preferable to DRI or UV detectors in the operation of LCs under limiting conditions.

The primary differences between 'Limiting Conditions of Solubility' and Belenkii's 'Critical Condition of Adsorption' approach are: the use of a thermodynamically poor (bad) eluent, or even an eluent which is a non-solvent for the polymer, while the polymer is dissolved and injected in a thermodynamically good solvent. The limiting condition method offers an advantage relative to Belenkii's approach since it is experimentally less demanding:

-the sample dissolution is faster
-the determination of the appropriate eluent composition is easier
-'limiting conditions" are less sensitive to slight changes in the eluent composition and temperature as well as to the presence of impurities, particularly water traces in the eluent
-"limiting conditions" can be used for high molecular weight polymers (up to 1,000,000 g/mol), whereas critical conditions are, thus far, limited to the characterization of macromolecules up to 100,000 g/mol.

Both approaches are accomplished isocratically and therefore the problem of irreproducible gradient production, which is a limitation for the quantitative analysis of copolymers by gradient chromatography, is avoided.

Applications of Limiting Conditions: Combination of SEC, Precipitation and Liquid Adsorption Chromatography

A limiting condition based method for the characterization of the molecular weight distribution of one component of a binary polymer blend has been developed where both polymers co-elute. In this technique a polymer mixture is dissolved in a good solvent for <u>both</u> constituents

and is injected into an eluent which is a strong non-solvent for one component of the blend. Examples have included the separation of polystyrene from either polymethylmethacrylate (PMMA) or polyvinylacetate over bare silica gel using toluene/methanol, THF/water and THF/n-hexane as the mobile phases. In these cases, the polystyrene elutes under a size-exclusion mechanism with its molecular weight distribution correctly estimated. The second polymer (PMMA or polyvinylacetate) elutes at its limiting condition. Its molecular weight distribution can also be resolved, if desired, with the addition of a second column. This is demonstrated in Figure 1.

POLYMER \ M	$M_W\ 10^{-3}$	$M_n\ 10^{-3}$	M_W/M_n
PS II. Column: PL-gel	432	348	1.24
PS II. Column: SI-1000, THF	429	345	1.24
PS II. + PMMA Column: SI-1000, THF	391	251	1.55
PS II. Column: SI-1000 THF:Hexane 82:18	424	346	1.23
PS II. + PMMA Column: SI-1000 THF:Hexane 82:18	453	368	1.23

inject →

V_R [ml]
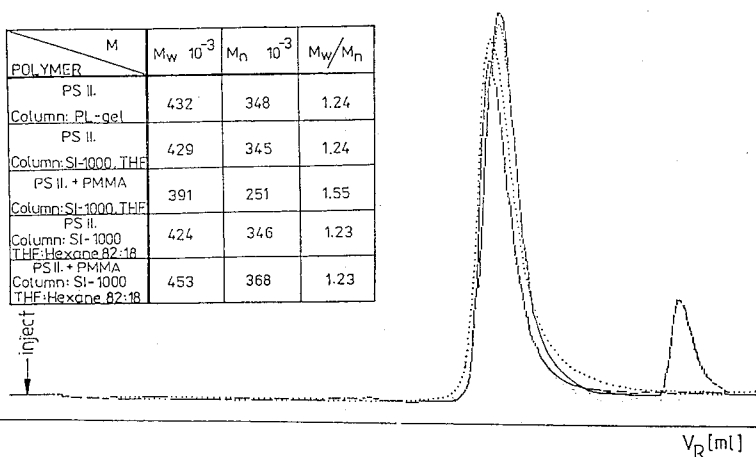
Figure 1: Typical chromatograms of polystyrene injected alone (-.-) and in a mixture with PMMA (...) onto a silica gel column with THF. Polystyrene injected alone (...) and with PMMA (___) under limiting conditions with THF/Hexane as an eluent 82:18 vol%. The table lists the calculated Mn and Mw data. For comparison the molecular weights determined for polystyrene on a PL-gel column are also tabulated. It is evident that the presence of PMMA lowers the apparent Mn and Mw of the polystyrene and artificially raises PDI when operating under SEC conditions. However, the injection under limiting conditions provided a very good agreement in the calculated molecular weights compared to the injections with polystyrene in isolation.

HPLC CHARACTERIZATION OF REACTION MIXTURES OF ACRYLAMIDE AND QUATERNARY AMMONIUM CATIONIC MONOMERS

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Copolymers of acrylamide with dimethylaminoethyl acrylate (DMAEA) and dimethylaminoethyl methacrylate (DMAEM), both quaternerized with methyl chloride, have been synthesized by inverse-emulsion polymerization. This involved the dispersion of the aqueous comonomer solution in a continuous organic phase (Isopar-M is a narrow cut paraffins with properties similar to decane). The inverse laticies were stabilized using low levels of block copolymeric surfactants based on polyethyleneoxide and poly-12 hydroxystearic acid. The polymerizations were chemically initiated using oil soluble azo species and were performed in jacketed stainless steel 5-Liter reactors computer controlled with respect to temperature. The reaction mixtures were continually agitated and sparged with nitrogen throughout the polymerization.

The characterization of the reaction mixture using an HPLC method will be discussed. The optimization of the stationary phase involved the comparison of two reverse-phase columns (C_{18} and CN). The mobile phases investigated included binary mixtures of methanol-water and acetonitrile-water with varying buffers and acids to adjust the pH. It was found that a 50:50 vol% mixture of acetonitrile-water provided the best peak shape. Additionally, an optimum pH of 3.0 was reported when the pH was adjusted with phosphoric acid. Dibutyl amine was found to be a good additive to compete with the quaternary ammonium monomers for surface adsorption and reduce the retention volume of the charged monomers. Figure 1 shows an example of a chromatogram generated under optimal conditions.

The information on the drift in residual monomer composition with conversion has been used to implement semi-batch policies to control the copolymer composition. Results will be shown where both monomers and only the more reactive monomer were fed as a function of time into the reactor. These policies were made more difficult by the non-standard behavior of the copolymerizations. For example, the reactivity ratios are functions of monomer concentration, pH, the ionic strength of the reaction mixture and the extent of the reaction. Figure 2 shows a plot of the comonomer composition as a function of conversion for both batch and semi-batch reactions. It is clear that the feeding of monomer as a function of time can be used to produce a high solids final product with a more uniform distribution of charged groups along the polymer backbones.
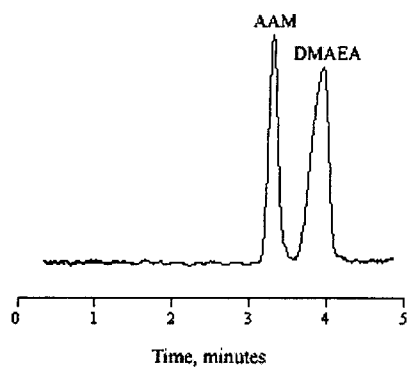
Figure 1 A typical chromatogram for the system acrylamide/dimethylaminoethyl acrylate (AAM/DMAEA). The peaks correspond to a concentration of 100 and 50 ppm of AAM and DMAEA, respectively. Conditions: 50:50 vol % acetonitrile/deionized water, 0.01 mol/L of dibutyl amine, pH=3.0 (phosphoric acid). Flow: 2.0 ml/min. Injection: 100 µL. Detection: UV, at 214 nm.
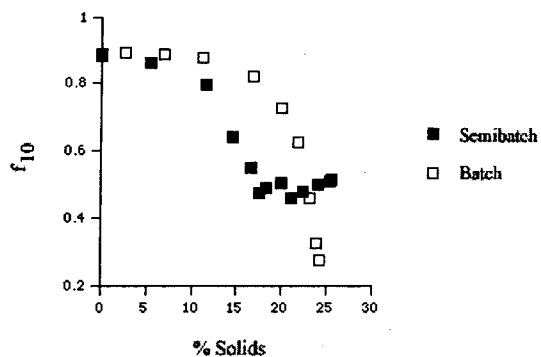
Figure 2 Comonomer composition as a function of percent solids for the inverse microsuspension copolymerization of unpurified AAM/DMAEM at 50°C using a copolymeric surfactant. Experimental conditions: [Monomer]=5.92 mol/L_w, [AIBN]=3.22 mMol/ L_o, f_{10}=0.89 and $\Phi_{w/o}$=0.74.

HPLC CHARACTERIZATION OF ACRYLAMIDE DIRECTLY INVERTED FROM A HETEROPHASE (INVERSE-EMULSION) REACTION MIXTURE

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A rapid and low cost method has been developed for the analysis of the residual acrylamide concentration from an inverse-emulsion reaction. Inverse-emulsions involve the dispersion of a water soluble monomer solution in a continuous organic phase. The addition of a low-medium HLB steric stabilizer and continuous agitation is required to maintain emulsification. One analytical procedure for the determination of residual monomer involves breaking the emulsion by centrifugation, decanting the organic phase and the subsequent redissolution of the monomer-polymer mixture in a purified aqueous solvent. This last step can require several days for the high molecular weight polyacrylamides (over 10 million daltons) to redissolve. Other methods are based on the precipitation of the polymer in a non-solvent; the polymer is separated by centrifugation and aliquots of the supernatant liquid are injected into a liquid chromatograph. The disadvantage of these methods is the use of expensive of mobile phases. In an attempt to overcome these limitations, several high HLB inverting surfactants were investigated. It was found that Tergitol TMN-10, (Union Carbide Chemicals, manufactured through the reaction of 2,6,8-trimethyl-4-nonanol with ethylene oxide, CAS Number: 60828-78-6) was the most suitable. The inversion procedure therefore involved adding 0.0050 grams of the reaction mixture to 20 ml of a water-surfactant solution under strong agitation. Aliquots of this inverted mixture were then injected into the liquid chromatograph. This consisted of a short Shodex OHpak SB-800P column (5 cm) as a stationary phase, a Hitachi L6000 pump and a Hitachi L4000H UV detector operating at a wavelenght of 214 nm. A micellar mobile phase was used which consisted of highly deionzed water with 25 mmol/L of electrophoresis grade sodium dodecyl sulfate (SDS). The SDS at a level above its critical micelle concentration, was used to solubilize the organic phase and emulsifier present in the water-in-oil (inverse) emulsion. These micelles then eluted from the column at very low retention volumes and did not interfere with the monomer peak. The large pore volume of the sorbent was required to separate the polymer from the residual monomer as is shown in Figure 1. From this figure two peaks are noticeable, a broad micellar and polymer peak at 0.5 minutes and a large and sharp acrylamide peak at 2.05 minutes. This method resulted in linear calibration curves for the acrylamide monomer up to 100 ppm as is shown in Figure 2. Further, the elimination of an organic mobile phase, reduced the cost considerably as is illustrated in Figure 3. In addition, the sample preparation time is reduced from 30 minutes to several days in the traditional methods to 15 minutes for the newly proposed method. Therefore, this inversion procedure is suitable for rapid data analysis from reaction mixtures obtained from inverse-emulsion polymerizations.
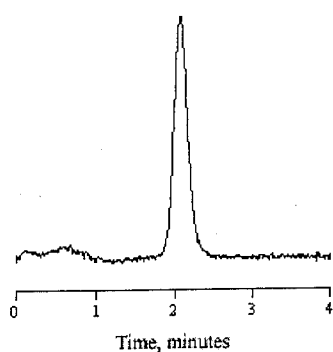
Figure 1 A typical chromatogram of an inverted wa-
ter-in oil acrylamide/polyacrylamide sample. The left
peak is the polyacrylamide with micellar solubilized
oil. The peak at higher retention times corresponds
to the acrylamide monomer. Conditions: 0.5 ml/min
and 214 nm.
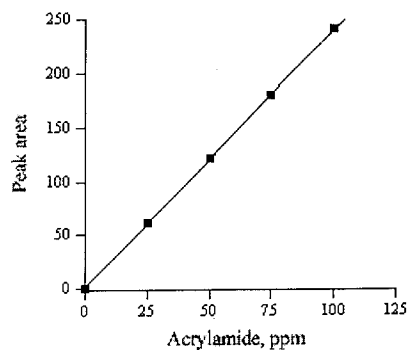
Figure 2 HPLC calibration curve for this method
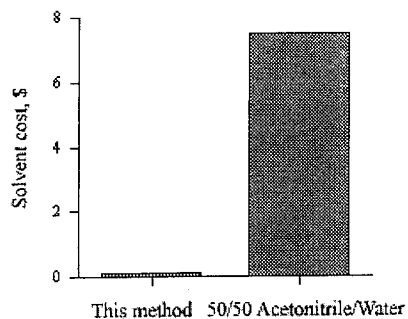
Figure 3 A cost comparison, based on 100 injections of
this method and another method which uses 50/50
acetonitrile/water. The column costs are comparable.

SEC OF HUMIC SUBSTANCES FRACTIONATED BY MODIFIED IHSS DISSOLUTION/PRECIPITATION METHOD FROM SLOVAK SOILS

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The organic matter of soils, peats and waters consists of a mixture of plant and animal products in various stages of decomposition together with substances synthesized biologically and/or chemically. This matter contains non-humic (relatively simple) compounds and humic substances (HS, not well defined oligomeric and polymeric compounds). HS's are usually divided by dissolution/precipitation procedure into three main fractions - humic acid (HA), fulvic acid (FA) and humin. The intention of our work is to characterize these fractions obtained according to modified IHSS (International Humic Substances Society) method by SEC on Sephadex gels. The high structural complexity and the wide range of molecular masses of humic substances are responsible for higher uncertainity in the interpretation of the results than is usual in SEC of other polymers. A number of aspects of SEC operational conditions (influence of pH, ionic strength, nature of buffer, sorption phenomena etc.) is not well understood till now due to variable ionic nature and complexity of functional groups in the structure of these polymers.

Our work is contributing to the knowledge with respect to systematic studies of the influence of pH, ionic strength , nature of buffer and counterion onto the SEC profiles and aromaticity (A4/6) of humic substances derived from Slovak soils of various origin.

This work is part of the studies on analytical methods for analysis of mercury and organomercurials in environmental samples granted by Slovak Grant Agency Project No.1/1640/94.

COMBINATION OF SEC AND FULL ADSORPTION / DESORPTION APPROACHES FOR MOLECULAR CHARACTERIZATION OF CONSTITUENTS OF POLYMER MIXTURES

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Owing to the wide application of polymer mixtures in recent years, the rapid determination of the M and MMD of their constituents becomes more and more important.

The elegant and straightforward separation of chemically different polymers having similar molecular dimensions offer coupled liquid chromatographic methods, e.g. "Orthogonal chromatography" [1], "On-Off elution method" [2], "Liquid chromatography at the critical point of adsorption" [3] or "Liquid chromatography under limiting conditions of solubility" [4].

Recently we suggested and tested a method of separation of polymer mixtures with different polarity based on combination of size exclusion and full adsorption (SEC/FA) [5]. This approach is extended in present work where the combination of size exclusion and full adsorption/desorption (SEC/FAD) mechanisms is applied. The experimental arrangement has been proposed and tested in which one column is used for temporary trapping of one constituent of a polymer mixture (FAD column) while another column (or a set of columns) is used solely for a size exclusion chromatographic separation (SEC column). In the first step, the more polar constituent of mixture is fully retained on the surface of the FAD column packing from a nonpolar eluent. Under the same conditions, however, the less polar constituent is not retained and passes into the SEC column where its M and MMD are determined. In the second step, the more polar constituent of the polymer mixture is desorbed from the FAD column using an appropriate desorbing eluent. The desorbed polymer is carried directly into SEC column where its M and MMD are determined, too. This SEC/FAD method permits separation of polymer mixtures and determination of M and MMD of their both constituents in the same apparatus in two successive steps. However, the FAD column can influence obtained chromatographic data and the resulting SEC peaks are shifted and broadened or even skewed. We will show that appropriate optimisation of both geometry and packing of FAD column solves these problems.

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2-DIMENSIONAL CHROMATOGRAPHY FOR THE DEFORMULATION OF COMPLEX COPOLYMERS

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Introduction:

The characterization of modern high performance polymers is still a challenge for polymer scientists. Copolymers and complex polymer blends play an important role in many applications. A fast, reliable and comprehensive method is needed to succeed in this task. Size-exclusion chromatography (GPC) is a standard method for the determination of molar mass distributions and molecular weights, if proper calibration has been performed. Since its early days, efforts have been made to use GPC in the characterization of copolymers [1],[2],[3]. Since GPC separation is not based on molar mass but molecular *size*, analysis of complex samples like copolymers and polymer blends modified by additives, plasticizers and various stabilizers by GPC alone is generally not sufficient.

The versatility of the 2-dimensional approach will be illustrated with a 4-arm star-shaped block copolymer (a mixture of 16 components), which was synthesized in our labs to understand and demonstrate the advantages of 2-dimensional chromatography. These 16 components are a mixture of four different styrene/butadiene (St/Bd) copolymer compositions each consisting of four molar masses (the St-Bd precursor with one to four arms).

Theory:

The main feature of polymers is their molar mass distribution that is well known and understood today. However, there are several other properties in which the breadth of distribution is important and which influence polymer behavior. These include:

- physical: the classical chain-length distribution
- chemical: two or more comonomers are incorporated in different fractions
- topological: polymer architecture may differ (e.g. linear, branched, grafted, cyclic, star or comb-like, dendritic)
- structural: comonomer placement may be random, block, alternating, etc.
- functional: distribution of chain functions (e.g. all chain ends or only some carry specific groups)

The main disadvantage of GPC is its inability to quantitatively distinguish different polymer architectures and chemical heterogeneity. Gradient HPLC has been useful for the characterization of copolymers [4],[5],[6],[7],[8],[9]. In such experiments careful choice of separation conditions is a *conditio sine qua non*. Otherwise, low resolution for the polymeric sample will obstruct the separation. On the other hand, the separation in HPLC, dominated by enthalpic interactions, perfectly complements the entropic nature of the GPC retention mechanism in the characterization of complex polymer formulations.

However, HPLC sorbents also show GPC behavior to some extent depending on the pore size of the stationary phase relative to the molar size of the solute. Copolymers with the same composition but different molar masses will in general have somewhat different retention characteristics. This may lead to copolymer HPLC fractions with heterogeneous chemical compositions, and may contain some chains with different molar mass *and* comonomer content.

We have tried to combine the advantages of HPLC and GPC by using a fully automated, software controlled, 2-dimensional chromatography system for the on-line analysis of composition, end-group functionality and molar mass distribution. It consists of two chromatographs, one which separates by chemical composition (e.g. a gradient HPLC) and a GPC instrument for subsequent separation by size.

Results

A very complex 16-component styrene/butadiene star block copolymer mixture was injected into a gradient HPLC (THF/i-octane) on a Silica column (60Å pore size) in order to get a good separation by chemical composition. These homogeneous fractions were transferred automatically by an injection valve into the second dimension (GPC in THF).

In a GPC separation a tetramodal molar mass distribution of the sample is observed, indicating the different arms of the star branched block copolymer. However, there is no sign of any difference in chemical composition, which actually varied from 20% to 80% of butadiene content.

Running the same samples in gradient HPLC alone also gives poorly resolved peaks, which indicate changes in composition but give no hint of different molar masses and structures. The combination of the two methods dramatically increases the resolution of the separation system and gives a clear picture of the complex nature of the sample mixture. Fig. 1 shows the contour plot of the 16-component sample. It is the result of 28 transfer injections from the gradient HPLC into the GPC part of the 2D system.

16 Component St-Bd Star Block Copolymer
(Contour Plot)

Fig. 1: 2D HPLC-SEC separation of a 16-component mixture of a star block copolymer (4 molar masses with 4 different St/Bd ratios each)

The contour plot clearly reveals the chemical heterogeneity (y-axis, chemical composition) and the molar mass distribution (x-axis) of the test mixture. The relative concentrations of the components are indicated by lines. 16 major peaks are resolved with high selectivity. These correspond directly to the components in the sample mixture. Some by-products are also revealed.

The high resolution of LC-GPC separations and the full automation using 2D-CHROM software enable the reliable and comprehensive characterization and deformulation of complex analytes like copolymers, polymer blends and additiv analysis.

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GPC ANALYSE OF CELLULOSE TRICARBANILATES FROM TENSION AND OPPOSITE BEECH WOOD

D. Baloghová, F. Kacík and Šindler

STUDY OF POLYMERS CONTAINING MICRODISPERSED CROSSLINKED PARTICLES BY HYDRODYNAMIC AND GEL PERMEATION CHROMATOGRAPHY METHODS

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In course of study of branched polymerization we discovered for the first time effects of separation according to the sizes of crosslinked polymer microparticles in GPC columns in organic solvents. Microparticles with sizes more then sorbent pores are separated according to the hydrodynamic chromatography principle. Method of registration of microparticles was suggested. Calibration connecting the diameters of microparticles with their retention volumes was obtained. These results have been used to study the size and content of microparticles in polymers, the structure of microdispersed network copolymers buit acording to the following principle: elastomer nucleus-rigid cell, the structure of impact-resistant polymethyl methacrylate. The content of crosslinked and noncrosslinked polymer in latex particles of polybutyl acrylate and in copolymer obtained by grafting of methyl methacrylate to crosslinked polybutyl acrylate by emulsion polymerization method was found. The degree of grafting was evaluated.

CHARACTERIZATION OF TECHNICAL WAXES USING CHROMATOGRAPHIC TECHNIQUES AND MALDI - MS

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Technical waxes as well as low molecular poly(ethylene)s were investigated using supercritical fluid chromatography (SFC), matrix-assisted laser desorption / ionisation - mass spectrometry (MALDI-MS) and size exclusion chromatography (SEC).

SEC provides for the determination of molar mass up to high values and simultaneously for molar mass distributions. The resolution of homologous species, however, is poor.

Especially in the low-molar mass range the use of SFC is advantageous in this respect. Proper resolution of homologes may be provided up to $M_W \sim 1000$ Da without greater difficulties.

In the last years MALDI-MS was established as a powerful technique for the determination of the mass of large biomolecules and even synthetic polymers. The ionisation of compounds using MALDI-MS is normally facilitated by the attachment of alkali metal ions of a aromatic matrix to the heteroatoms of the investigated compounds. Nonpolar hydrocarbons give no results under these conditions of ionisation, because of the lack of these sites. The addition of silver salts overcomes these problems.

In the reflectron mode excellent MALDI mass spectra could be achieved in the molar mass range up to 1000 Da. The determination of molar masses and mass distribution was in good agreement with the results of SFC. Using linear mode molar masses could be determined to nearly 3000 Da.

Beyond this mass range SEC remains yet the method of choice.

Advantages and difficulties of the used methods are discussed in this paper.
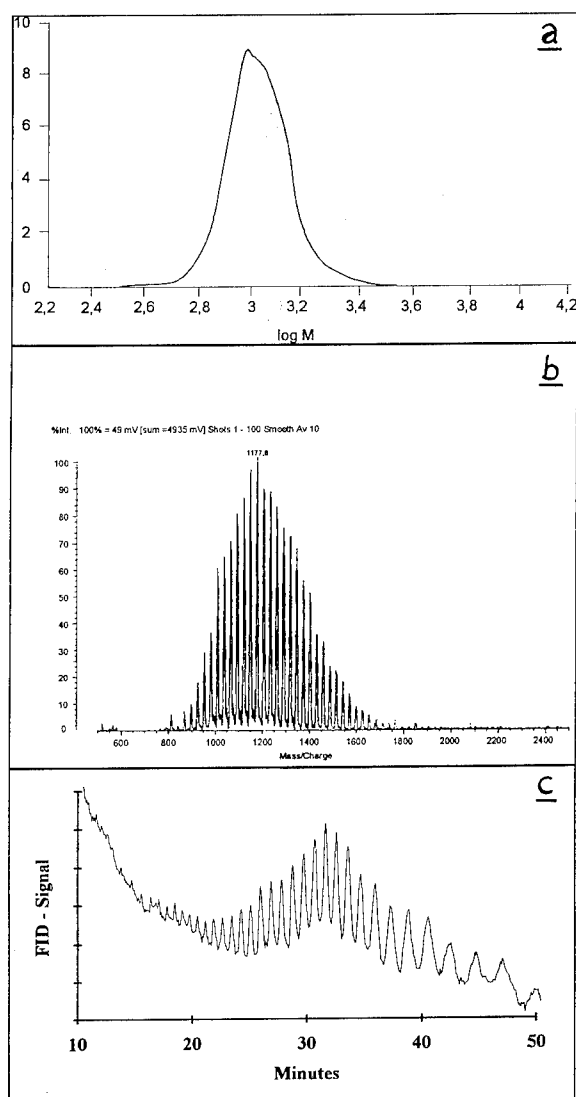
Fig.1: SEC-, SFC-chromatograms and MALDI mass spectrum of a technical wax
(Mw ~ 1000 Da)

MOLECULAR CHARACTERIZATION OF HOMOPOLYMERS AND COPOLYMERS BY COUPLING MULTIANGLE LASER LIGHT SCATTERING AND SEC

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We have studied in a number of poly(methacrylates) and poly(itaconates) the influence of the chemical structure of the repeating unit on its conformational parameters. Most of the unique properties exhibited by polymers are a result of the quasi–unlimited number of spatial arrangements that long chains can assume. To understand the factors involved in the flexibility or rigidity behaviour in a series of polymers, it is necessary a good molecular characterization and to be able to evaluate the interactions contributions of the lateral chains. In some polymer systems, the molecular characterization is complicated and we have considered to work by coupling Multiangle Laser Light (LS) and size exclusion chromatography (SEC).

The results obtained by this combination of on line LS detection and SEC separation is discussed. The nonexclusion effects in polymers containing long side chains are explained in terms of specific and hydrophobic interactions.

APPLICATION OF TLC IN COMBINATION WITH MICROCOLUMN CHROMATOGRAPHY IN THE ANALYSIS OF POLY- AND OLIGOSTYRENES WITH FUNCTIONAL GROUPS

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A possible method of obtaining polymers with a complex architecture involves the interaction between living poly- and oligostyryl lithium chains with methyl acetate and methyl benzoate, which leads to functionalization and crosslinking of macromolecules [1]. A detailed analysis of products of this reaction requires a combination of different chromatographic methods, ensuring sample fractionation with respect to both molecular weight and functionality. In the present work thin-layer chromatogrphy (TLC) was combined with high-performance size-exclusion microcolumn chromatography (MEC) for analyzing complex reaction mixtures.

The reaction products were separated into components according to the types of functional groups on analytical TLC plates, coated with KSKG silica gel and a silicic acid sol as a binder. The fractions removed from silica gel in the amount of 0.1 mg each were analyzed by MEC on a "KhZh-1309" chromatograph. Fluoroplastic microcolumns with 0.5 mm i. d. packed with Silpearl (for oligomers) or Separon (for polymers) were used. Functional groups were identified by NMR and IR spectroscopy.

In order to extend the diagnostic possibilities of TLC under the critical conditions, the effect of the polystyrene chain length and the sorbent pore width on the chromatographic behaviour of polystyrenes with functional groups was investigated.

A combined use of TLC and MEC made it possible to show with minimum time and labour expenditure that the reaction proceeds by a more complex mechanism than that suggested previously [1]. These two methods are adequately combined with respect to scales, ensure high efficiency, simplicity and speed of analysis, and require minimum consumption of sorbents, plates and solvents.

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NEW ASPECTS IN THE INTERPRETATION OF ANALYSIS OF COPOLYMERS BY MEANS OF MULTIDETECTOR GPC METHOD

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At present time the method of Gel Permeation Chromatography based on two coupled detectors for determination of copolymers composition is widely used. This method is rather simple, but there are some unsolved problems with interpretation of analysis data.

Some authors underline factors which cause main influence on the analysis data: shift between peaks due to the presence of conjunctive capillary between detectors, peak widening in this capillary, non linearity of concentration dependence of detector homopolymer signal and influence of common parameters of chromatographic system. In case of determination of mean copolymer composition we achieve good results, but composition curve will be incorrect.

Determination of composition inhomogeneitty of copolymers in our case was carried out by means of two methods: by mathematical modelling of composition determination and experimentally, using Gel Permeation Chromatography of known polymer mixtures.

Using the model which is based on the method of doubled detection of data representing Gaussian distribution with various parameters, we show the influence of distribution width and shift between peaks on the shape of composition curve.

Using model system PS-PDMS it is shown that for PDMS content less than 20%, error of mean composition determination increases sharply.

It is found that hypothesis of linear dependence detector response for the copolymers versus its composition does not work. The reasons of this phenomena are discussed.

GPC AND HIGH TEMPERATURE GPC OF POLYMERS ON A GIGAPOROUS SILICA GEL COLUMN PACKING

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Some polymers have extremely high molar mass, i.e. more than 10^7 g/mol. At present, only a few column packings based on polystyrene/divinylbenzene gel (Styragel, Waters, Milford, USA; PL-gel, Polymer Laboratories, Church Stretton, England) are available for GPC separation of such polymers. A disadvantage of these organic column packings are the limited options for the choice of the solvents. Moreover, these organic column packings have limited long time stability, when used for high temperature GPC measurements. On the other hand, normal phase silica gel column packings are almost universally usable with many different solvents. Such anorganic column packings with exclusion limits of more than 10^7 g/mol are, however, at present not available on the market. This is mainly due to the lack of mechanical stability usually observed with large pore silica gel materials.

We have tested the silica gel packing with very large pores prepared in the Polymer Institute, Slovak Academy of Sciences, Bratislava [1]. The particles with average diameter 10 um were packed into 25x0.8 cm columns. Its calibration curves in different solvents showed exclusion limits higher than available polystyrene standards, i.e. more than 2.10^7 g/mol. The results of the chromatographic tests, including a study of shear degradation of polymers at room and high temperature in different solvents and of adsorption of polymers on these columns will be presented.

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QUANTITATIVE DETERMINATION OF ANTIOXIDANTS IN POLYETHYLENE AFTER ITS DISSOLUTION IN AN AUTOCLAVE

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The physical properties of polymers strongly depend on the content of additives which they contain. The concentration of some additives, especially antioxidants and UV stabilizers, may change with time due to their diffusion, degradation or chemical reaction with other components. Therefore, the knowledge of the concentration of the additives is important both from the practical and theoretical point of view.

We have developed a new procedure for the analysis of the antioxidants within polyolefins: The polyethylene sample is dissolved in pure n-heptane or in a mixture of n-heptane and polar aliphatic modificator. The dissolution requires an autoclave (temperature 160-170°C, pressure about 5-10 atm). The time necessary for the dissolution of polyethylene granulate is determined in a separate experiment using a modified autoclave with integrated glass windows. The n-heptane is UV-transparent, what allows that after precipitation of the polymer by cooling to room temperature, the supernatant may be directly injected into a HPLC system equipped with an UV-detector. The normal phase, isocratic HPLC is then used for determination of the concentration of the antioxidants in the supernatant.

In the case that the concentration of an additive is too small for the detection, the solution was preconcentrated by partial evaporation of n-heptane at room temperature.

The applicability of the above procedure was first demonstrated by the analysis of the phenolic antioxidants Irganox 1010, Irgafos 168 (both of Ciba-Geigy, Basel, Switzerland), and alpha-tocopherol [1]. Recently, we have improved the experimental assembly and applied the described procedure to the analysis of the thiophenolic antioxidant Santanox R (Monsanto, Brussels, Belgium) and the UV-stabilizers Tinuvin 622 and Chimasorb 944 (both of Ciba-Geigy, Basel, Switzerland).

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** in partial fulfilment of the requirements for a Dipl. Ing. Thesis at Montanuniversität Leoben

SEC OF THERMOPLASTIC POLYURETHANE ON A SILICA GEL COLUMN WITH N-METHYL-PYRROLIDONE AS ELUENT

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The molar mass distribution of thermoplastic polyurethane (TPU) samples is often characterized using N,N-dimethylformamide as an eluent. We have obtained a new type of thermoplastic polyurethane, which was insoluble in this solvent but completely soluble in N-methyl-pyrrolidone. Polystyrene (PS) standards are also well soluble in this solvent, which can be used as mobile phase in SEC on normal phase silica gel columns at room temperature; a column 25x0.6 cm packed with 10 um silica gel particles prepared in the Polymer Institute, Slovak Academy of Sciences, Bratislava was used. The composition of the eluent was monitored with a refractive index (RI) detector.

The intrinsic viscosities of these TPU samples and of PS standards were also measured.

A new method for the calculation of the unknown Mark-Houwink constants for this series of TPU samples in N-methyl-pyrrolidone was developed, which needs only SEC data of a set of TPU samples and of polystyrene standards and the values of the intrisic viscosity of the respective sample set.

With these data, the molar mass distributions of a series of similar TPU samples were calculated by assuming the validity of the universal calibration.

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APPLICATION OF MULTIDETECTOR GPC TO STUDIES OF E-BEAMED POLYETHYLENE: A NEW LOOK

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The responses of polymers to radiation, and in particular electron beam radiation, have been extensively studied and reported. Numerous analytical techniques have been utilized in these experiments to determine crosslink yields, gel fractions, scission, etc. Gel permeation chromatography (GPC) is one of those techniques. Recent developments in multidetection techniques, however, hold promise for providing additional information and detail to studies of beamed polymers. The combination of viscometer and differential refractive index (DRI) detection enables the determination of "standardless" molecular weights. Branching indices can be determined for the soluble fractions as well as the viscosity distributions. Viscometry is highly sensitive to high molecular weight fractions present at low concentrations. Concentration detectors such as DRI's often miss these fractions. This information was not obtainable until recently and is not widely available for new polyethylene materials. This paper describes our work using multidetection HTGPC for the study of beamed polyethylenes.

TACTICITY DISTRIBUTION OF POLYPROPENE BY PREPARATIVE AND ANALYTICAL TREF

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It is known that heterogeneous Ziegler-Natta catalysts used in poly-α-olefins production are characterized by the presence of a plurality of active species, each having its own kinetic constants and stereospecificity.

Thus, a complete characterization of the polymer requires its separation into homogeneous fractions and subsequent microstructural analysis through ^{13}C NMR [1-8]. Several polypropene samples synthetized with different catalytic systems based on $TiCl_4$ supported on activated $MgCl_2$ with different electron donors were studied using both preparative and analytical TREF.

As expected, the ^{13}C NMR and DSC analyses of the key fractions show that temperature rising elution fractionation (TREF) separates polypropene mainly according to its tacticity. Thus, in principle, if we have both the same type and distribution of defects, along with a negligible effect of molecular weight, a single mastercurve can be obtained by plotting a ^{13}C NMR parameter related to the polymer stereoregularity (e.g. the pentad content) or a thermal property, such as melting point, vs the elution temperature. Once this has been verified, one can replace the cumbersome and time-consuming preparative TREF (P-TREF) with the more rapid analytical TREF (A-TREF).

The TREF analyses were focused on the most stereoregular polymer component.

The experimental results show that each variation in the catalytic system can be distinguished by both the P-TREF and A-TREF polymer dissolution profiles, which clearly reflect the nature of the parent catalyst system.

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INVESTIGATION OF GRAFTED IMPACT POLYPROPYLENE BY TEMPERATURE RISING ELUTION FRACTIONATION

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Abstract:

The goal of the presented work is the investigation of a two-phase, impact-resistant polypropylene (I-PP) by temperature rising elution fractionation (TREF). The polypropylene is a commercial product from BASF AG Ludwigshafen, which contains approximately 45 wt.-% dispersely distributed ethylene-propylene rubber in a homopolymeric PP-matrix. The production of such I-PP offers new possibilities for grafting these materials by means of extrusion technique. It is expected that the presence of a rubber phase ($Tg \ll R.T.$) in the semicrystalline matrix ($Tg > R.T.$) leads to a different grafting efficiency in both phases. Successfull characterization of the grafted products is possible only after complete separation of the rubber phase from matrix material. The separation can be carried out by TREF.

The poster shows the results of grafting reaction of maleic anhydride (MAN) onto I-PP. The primary object was to demonstrate the course of grafting reaction and the side reactions (degradation of the homopolymeric matrix, crosslinking of the rubber) in both phases of the impact-resistant PP. To compare the results, three different modified samples were separated into three fractions: at 60°C the amorphous rubber and at 128°C the homopolymeric matrix. The third fraction, separated at 96°C containes both ethylenic and propylenic structures with a high crystallinity.

The investigation shows that the grafting reaction occurs mainly in the amorphous rubber of the two-phase material. The separate grafting of the rubber phase has been shown to be possible at the minimum of radically degradation reaction of the homopolymeric matrix.

Hence, the combination of fractionation by TREF with other analytical methods (FTIR, GPC) allows to determine the course of grafting reaction in the two-phase polypropylene.

AFFINITY CHROMATOGRAPHY OF GLYCOENZYME AND GLYCOPROTEINS ON CONCANAVALIN A - BEAD CELLULOSE

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In the present work a conjugate of Concanavalin A (Con A) with macroporous bead cellulose was investigated as affinity chromatography matrix for glycoenzymes and extracellular glycoproteins. A specific sorbent Con A-triazine bead cellulose (Con A-TBC) was prepared from Perlose MT 100 (bead cellulose manufactured by Lovosice, Czech Republic). Con A was bound covalently to the cellulose triazine in a weakly acid medium (pH 5.8) at room temperature (1). The mild conditions of Con A immobilization were chosen to avoid impairment of its mannose/glucose binding activity. The prepared Con A-TBC was applied in affinity purification of glycoenzymes and glycoproteins. Enzymes as invertase from baker's, *endo*polygalacturonase (Rohament P) and exopolygalacturonase from carrot juice as well as extracellular mannan-glycoproteins from the yeast Cryptococcus laurentii (CL) were examined. Con A is lectin which has ability to interact with glycoconjugates containing mannose and/or glucose units (2). All glycoproteins used in present work fulfil this prerequisite. The chromatography experiments were performed in minicolumns filled with 1g of wet Con A TBC gel. The amount of immobilized ConA in affinity matrix was chosen considering the strength of interaction Con A : glycoprotein. It was found that optimum content of Con A for individual glycoproteins were as follows : 1.2 mg Con A/ml of gel for invertase, 5 mg Con A /ml of gel for *endo*- and *exo*polygalacuronase and 8 mg Con A /ml of gel for extracellular manno-glycoprotein CL. The minicolumn fillings were loaded with the solutions of above mentioned glycoconjugates which have contained the amount of the sample within the range 10-60 mg. The non-specifically adsorbed part of the sample was eluted with equilibration buffer and glycoconjugates specifically bound were eluted with solution of corresponding counter-ligand α-methylmannopyranoside (concentration 0.1-0.5 mol.l^{-1}). The purification of all glycoproteins hereby examined was remarkable. Individual purification factors estimated from measurements of specific activity of crude and purified glycoenzymes were 10x for invertase, 93x for *endo*polygalacturonase and 50x for *exo*polygalacturonase. The yeast mannanprotein was isolated from the extracellular heteroglycoprotein fraction. The monosaccharide component of purified mannanprotein was mainly mannose, with traces of glucose. The glycoenzymes and the yeast mannanprotein were homogeneous on FPLC chromatography.

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SUPRAMACROMOLECULAR KRAFT LIGNIN COMPLEXES

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Lignins. In constituting the second most abundant group of biopolymers, lignins are found in the cell walls of all vascular plants and woody tissues; their biodegradation is considered to be the rate-limiting step of the carbon cycle. The final stage in lignin biosynthesis involves the enzyme-catalyzed dehydrogenative polymerization of three monolignols, *viz. p*-coumaryl, coniferyl and sinapyl alcohol (*1*). The resulting macromolecules are said to embody about 10 different interunit linkages, half of which are the same β-*O*-4 alkyl aryl ether type (*2*).

Even though their configurations can vary with plant species, cell type and morphological region of the cell wall where they occur, lignins are generally thought to be assembled without regard to specific sequences of structural features along the polymer chains. Quite recently, however, it was observed that lignin macromolecules can promote, through noncovalent interactions, the formation of high molecular weight species during the dehydropolymerization of coniferyl alcohol *in vitro* (*3*). Thus the possibility of direct macromolecular replication during lignin biosynthesis has arisen for the first time.

Kraft Lignins. The majority of linkages between the monomer residues in lignins are much more stable than those in most other biopolymers. Consequently the conditions required for isolating lignins from plant and wood cell walls usually engender substantial degradation of these rather inert macromolecular chains.

Well over 40 million tons of kraft lignin derivatives are produced annually in the world as a byproduct of converting wood chips into pulp for manufacturing paper. Having been formed under quite severe conditions (typically during 2 h at 170°C in aqueous solution containing 45 gL^{-1} NaOH and 12 gL^{-1} Na_2S), kraft lignins are thought to have undergone major structural modifications compared with the native biopolymer (*4*); they are commonly viewed as being almost hopelessly complicated mixtures of degraded and partially "condensed" components.

Association between Kraft Lignin Components. The physicochemical properties of kraft lignins are profoundly influenced by noncovalent interactions between the constituent molecular species (*5*). In aqueous alkaline solutions, the resulting associated macromolecular complexes appear to be assembled in a very specific way: each behaves as though it possesses a single locus which is complementary to only one of the various individual components present in the sample (*6*). Thus kraft lignins seem to embody the reflection of a native biopolymer which is configurationally far from random.

Supramacromolecular Kraft Lignin Assemblies. The negative charge densities on the phenoxide moieties of the individual associating components impose limits upon the sizes that can be attained by macromolecular kraft lignin complexes in aqueous alkaline solution. Because they are not subject to such restraints, the dimensions of acetylated methylated kraft lignin complexes in DMF extend to magnitudes above those of 20 million molecular weight polystyrene components even though $\bar{M}_w$ for the *individual* species seldom exceeds 5000 (*7*). Yet the apparent molecular size distributions of these acetylated methylated kraft lignin derivatives in DMF are multimodal rather than continuous in form. Accordingly the supramacromolecular complexes must be assembled in a well-defined way that is ultimately governed by vital structural *motifs* originally embroidered within the framework of the native biopolymer.

Interconvertibility of Supramacromolecular Kraft Lignin Assemblies. The largest acetylated methylated kraft lignin species that appear in the size-exclusion chromatographic profiles resulting from elution in DMF through 10^7 Å pore size poly(styrene-divinylbenzene) seem to be interconverted in a rather specific way (Figure). During incubation of a 0.2 gL^{-1} acetylated methylated kraft lignin sample solution at 20.0°C for 146 h, the populations of supramacromolecular complexes corresponding (in their formal elution behavior) to 4×10^8 and 30×10^8 molecular weight polystyrenes steadily increase at the expense of the largest entities which emerge from the column as though they were 100×10^8 molecular weight polystyrenes. Although their true molecular weights have not been measured, these acetylated methylated kraft lignin assemblies may be presumed to be very large. Despite their size, they remain well-defined; evidently some aspects of the blueprint for the native biopolymer survive among the kraft lignin derivative components from which they are constructed.
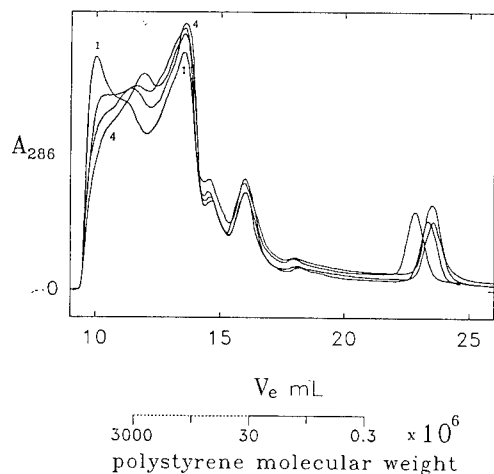
Figure. Dissociation of supramacromolecular assemblies during incubation of acetylated methylated kraft lignin derivative (8) in DMF at 0.2 gL^{-1} for (1) 0 h, (2) 23 h, (3) 77 h, and (4) 146 h at 20.0°C. Elution profiles from TSKgel®G7000-H6 column monitored at 286 nm.

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SEC AND DLS STUDY OF POLYMERIC COMPLEXES

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INTRODUCTION

The phenomenon of stereocomplex formation of isotactic and syndiotactic poly(methyl methacrylate) (i- and s-PMMA) has been much investigated in the past[1]. We believe that the recently developed high-resolution computerized techniques used in the present study could provide a more precise idea[2,3] of hydrodynamic behaviour and structure of stereocomplexes.

EXPERIMENTAL

The complexation took place in very dilute tetrahydrofuran solution using three PMMA stereoisomer pairs of different tacticity and chain length. PMMA stereoisomers were mixed in ratio i : s = 1 : 2 at which the intensity of complexation should be the highest[2]. After stabilization ($\sim$ 20 hours), aggregated systems were investigated by size exclusion chromatography (SEC) with refractive index and low-angle static light scattering detection as well as by dynamic light scattering (DLS).

RESULTS

Since SEC separation of PMMA stereocomplexes is controlled by their hydrodynamic volumes, $[\eta]M$, the resulting size distribution is real while the molecular weight distribution obtained simultaneously is rather apparent. The weight and intensity (z-fraction) distribution of radii of gyration, R_g, were calculated from SEC data using the Flory-Fox relation. This intensity-defined distribution of R_g was compared with the distribution of hydrodynamic radii of equivalent spheres, R_h, obtained from DLS experiment using CONTIN procedure and the Stokes formula. In addition to the average stereocomplex sizes and the reliable relations between R_g or R_h and molecular weight, the ratios of proper average radii R_g/R_h of stereocomplexes having different compactness are presented.

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CHARACTERIZATION OF POLYSTYRENE-POLY(ETHYLENE OXIDE) GRAFT COPOLYMER BY SIZE EXCLUSION CHROMATOGRAPHY AND LIQUID CHROMATOGRAPHY AT THE CRITICAL POINT OF ADSORPTION ON A POLYSTYRENE GEL

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Liquid chromatography under critical conditions (LCCC) and size exclusion chromatography have been applied to the analysis of the polystyrene-poly(ethylene oxide) graft copolymer.

The copolymer has been prepared by the dispersion copolymerisation of styrene and methacroyl terminated poly(ethylene oxide) macromonomer.

The size-exclusion chromatography measurements were performed using Phenogel (pore size = linear, $d_p=\mu m$, 7.8×300mm) column and tetrahydrofuran (THF) as the mobile phase. The critical conditions of adsorption for macromonomers were identified for THF/n-hexane (71/29, wt/wt) eluent and for polystyrene gel packed column (Phenogel, pore size = linear, $d_p=10\mu m$; 7.8×300mm). This means that the LCCC is not limited to the solid column packing like silica gels. Molecular weight distribution of polystyrene backbone was measured under critical conditions for the grafts formed by poly(ethylene oxide).

The copolymer was characterized independently by light scattering and results obtained were compared with chromatographic data.

SEPARATION OF CHEMICALLY HETEROGENEOUS COPOLYMERS BY SIZE EXCLUSION CHROMATOGRAPHY WITH DUAL DETECTION

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The combination of size exclusion chromatography (SEC) with an absolute method for molecular weight determination, e.g., light scattering (LS), brings new possibilities to the polymer analysis. On the other hand, theoretical problems arise in the evaluation of experimental data. This holds true especially in SEC of copolymers.

It is assumed that the separation process is governed by the size exclusion radius of the molecule, R_{SEC}, but there is no general agreement whether R_{SEC} corresponds to the theoretically well-founded mean projection on a line, the viscosity radius, or the radius of gyration[1]. On the other hand, as pointed out by Dubin et al.[2], if the SEC studies are confined to a single structural type, virtually any dimensional parameter will prove successful.

Our study is focused on the flexible chain copolymers of two comonomers, A and B, and the hydrodynamic volume, $[\eta]M$, will be taken as a measure of the molecular dimensions[3]. These may be conveniently expressed as the hydrodynamic volume which is for a given elution volume constant. For a homopolymer, it is given by the calibration, for a copolymer, it can be computed according to equations of classical hydrodynamics[4] rearranged for example in the form

$$\frac{[\eta]M}{\Phi_{0,\infty}} = G_1 M \left(1 + G_2 M^{\frac{1}{2}} \right)^{\frac{3}{5}} \tag{1}$$

where the functions G_1 and G_2 are given by

$$G_1 = \left(\frac{2l_u^2}{\overline{m}_L} \right)^{\frac{3}{2}} C_{\infty,Co}^{\frac{3}{2}} \tag{2}$$

$$G_2 = 1.9 \left(\frac{3\overline{m}_L}{4\pi l_u C_{\infty,Co}} \right)^{\frac{3}{2}} B_{Co} \tag{3}$$

where $\overline{m}_L$ is the average molecular weight per unit contour length, L, $[\eta]$ the intrinsic viscosity, M molecular weight, $B_{\infty,Co}$ parameter of interaction of polymer with solvent (average value), $C_{\infty,Co}$ characteristic ratio (average value), l_u monomeric unit length ($l_u = 1.5 \times 10^{-8}$ cm), $\Phi_{0,\infty}$ viscosity function.

The dual molecular weight—composition distribution function $w(M,x)$ was expressed as a product of two individual distributions, the first of molecular weight approximated by the Schulz-Zimm and Stockmayer functions[5], and the second by the
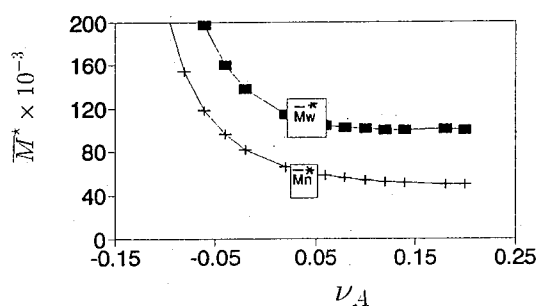
Figure 1: The apparent number- and weight-average molecular weights, $\overline{M}_n^*$ and $\overline{M}_w^*$, respectively, as functions of the refractive index increment of one component, ν_A (the increment of the other component was kept constant, $\nu_B = 0.2$, during the calculation)

function of Myagchenkov and Frenkel[6] as well as by the "rectangular" model (all compositions with the same probability) function.

The results may be summarized as follows:

1. Significant deviations of experimental molecular weight averages from the true ones due to the effect of composition heterogeneity may be expected for largely different refractive index increments[7] of both constituents (Fig. 1l).

2. In the case of "ideal detection", i.e., for equal refractive index increments of both constituents, the error due to chemical heterogeneity in experimentally obtained molecular weight averages is within the experimental error and may be neglected. A larger error appears at highly different values of the characteristic ratio of the constituents or of the interaction parameter B_{A,c_0}.

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SEPARATION OF PARTICLES BY GRAVITATIONAL FIELD-FLOW FRACTIONATION - ROLE OF SURFACE FORCES

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The chemical affinity cannot be often employed as the separation principle for separation/characterization of macromolecules or particles. Therefore methods based on other principles have to be used. Field-flow fractionation (FFF) is one of such methods. The separation effect is reached by simultaneous activity of a physical field (e.g., electrical, thermal) and a non-uniform flow velocity profile of the carrier liquid passing through a capillary space (the separation channel).

Gravitational field-flow fractionation (GFFF) is the experimentally simplest FFF technique where weight, the lift forces and surface (colloidal) forces determines the sample retention. In this report we demonstrated the influence of surface forces, i.e. electrostatic repulsive forces (caused by charged particles and channel walls) and van der Waals attractive forces (adsorption). We showed how quality of the separation channel bottom together with ionic strength of the carrier liquid can be employed for optimization of the separation process.

SYNTHESIS AND MOLECULAR CHARACTERIZATION OF BULKY SIDE CHAIN POLYMERS

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The effect of the side chain structure on the solution behaviour of vinyl polymers has been previously studied for a number of poly(methacrylates) and poly(itaconates). The conformational study of polymers containing aromatic, aliphatic and cyclic side chains has demonstrated that the nature, volume and structure of the side chain influence notably the conformation and rigidity of the polymer chain. The size and nature of the lateral groups play an important role in the dilute solution behaviour of these polymers and seems to be the driving force for the high rigidity. In this work we describe the synthesis, solubility, fractionation and molecular characterization by viscometry and multiangle laser light scattering of bulky side chain polymers such as poly(methacrylates) containing 2,4-dimethylphenyl (2,4DMP), 2,5-dimethyl phenyl(2,5DMP) and 3,5-dimethylphenyl (3,5DMP) groups and poly(itaconates) containing 1-indanyl (PM1I), 2-indanyl (PM2I) and 5-indanyl (PM5I) groups. Monomers were synthesized by reaction of methacryloil chloride with the corresponding phenols in benzene solution. Monoitaconates were obtained by reaction of itaconic acid with the corresponding indanols in the presence of acetyl chloride. Fractionation was achieved by fractional precipitation. The fractions were characterized by viscometric, osmometric and Size exclusion Chromatography and multiangle light scattering measurements. Viscosities at 298 K in different solvents were measured using a Desreux- Bischoff dilution viscometer and intrinsic viscosity $[\eta]$ was determined by the usual extrapolation of the reduced viscosity. Nonexclusion effects can be detected what is attributed to strong interactions due to polar groups.

Fractions are reasonably monodisperse. From the usual log-log plots of $[\eta]$ against M_w, the Kuhn-Mark-Houwink-Sakurada (KMHS) equation ($[\eta] = K_a M_w{}^a$) was established for 2,4DMP, 2,5DMP, 3,5DMP, PM1I, PM2I and PM5I in different solvents. We have also determined the conformational parameters K_Θ related to the unperturbed dimensions of the polymer chain, from data in good solvents. The thermodynamic parameter B, is in good agreement with those of the viscometric parameters K_a and a in the sense that high values of a and B are obtained in the best solvents and $a = 0.5$ and $B = 0$ for theta condition. The characteristic ratio C_∞ as defined by Flory is considered the best parameter for describing the steric hindrance of the polymer chain. This parameter has been calculated from experimental values of $(<r^2{}_0>/M)^{1/2}$. The conformational parameters increases as the volume of the side chain and steric hindrance increases. The high C_∞ values found for the polymers in this work indicate that the steric hindrance introduced in the side chain due to the substituents in the aromatic ring exerts a marked influence on the conformational parameters. These observations are in good agreement with rotational isomerization theory, applied to the study of other polymers with large substituents. Bulky substituents show important hindrances to internal rotation as it was demonstrated for other members of the family of aromatic poly(methacrylates). The rigidity factor is drastically influenced by the substitution on the aromatic group of the side chain. Two factors should be taken into account: interactions between aromatic rings and the bulkiness of the side group. These two factors are more or less important depending on the polymer structure and certain changes in the rigidity can be observed. The presence of substituents such Cl or $-CH_3$ or $-CH(CH_3)_2$, close to the main chain affects the internal rotation potential, and the internal rotation of the side groups should be more restricted than in the case of poly(phenyl methacrylate). A very noteworthy result is the rigidity factor obtained for 3,5-DMP which has the two *ortho* positions free of substituents and however shows the higher σ value. It is difficult to give an explanation but if we compare these results with those reported for chlorinated derivatives, we can observe that no clear correlation between bulkiness and σ is found. In conclusion, interactions between aromatic rings and the bulkiness of the side groups are two factors which play an important role in the rigidity of this kind of polymers.

MOLECULAR WEIGHT DISTRIBUTION OF STARCH POLYSACCHARIDES

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In recent years the development of starch based polymeric materials is of rising interest. Macroscopic properties of such materials strongly depend on specific molecular parameters of the basic polymers. The optimization of properties of starch based products like gels, films or formed materials requires the research of composition- and structure-function relationship of the starch polysaccharides. Knowledge of the extent and manner of depolymerisation is necessary to understand and to optimize degradation processes or chemical modification of starches.

Size exclusion chromatography (SEC) in combination with double detection of differential refractive index (DRI) and multi angle laser light scattering (MALLS) was used to investigate starch polysaccharides. Molecular parameters such as molecular weight distribution, molecular weight averages and radii of gyration can be determined by this technique.

Starch consists of two major polymers, the branched amylopectin and the mainly linear amylose. These two components are stored in plants in starch granules which have a highly ordered partly crystalline structure.

Complete dissolution of starch granule structure is necessary for molecular characterization. We solubilized the starch granules and isolatated starch polysaccharides in dimethyl sulfoxid (DMSO) at temperatures above 120°C to 160°C in N_2-atmosphere under stirring. The characterization of the two components amylose and amylopectin is the prerequisite for the investigation of mixtures of the polysaccharides like native or processed starches. Examples will be given for molecular parameters of amylose, amylopectin and mixtures thereof, starches from wheat, wrinkled pea, potato and extruded starch samples.

NEEDS FOR ROUND ROBIN TESTS IN SIZE EXCLUSION CHROMATOGRAPHY. EXAMPLE OF POLYAMIDES

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Size Exclusion Chromatography (SEC) is a powerful tool for a basic characterization of polymeric materials : the molecular weights distribution.

Although SEC is widely used, for example, in the following fields: macromolecular synthesis, production control, polymer ageing studies ; few efforts have been done, to our knowledge, for a standardization of methods [1]. This is probably due to two factors. On one hand, apart classical room temperature SEC in THF, most of methods are specific to a class of polymers or even more to individual ones. On the other hand, polymer manufacturers have a good knowledge of their polymers and develop proprietary methods that are considered confidential.

Nevertheless, it would certainly be interesting both for manufacturers and customers to involve in round robin test of methods devoted to technical polymers.

The example of High Temperature SEC characterization of polyamides will be given. The method used in our laboratory was developped on the basis of literature data [2] and is applied for characterization of samples used in petroleum environnement, mainly in off-shore oil production where flexible pipes including polyamides internal sheet in their structure are used. The experimental conditions of the method will be given with its intra-labory statistical evaluation. This statistical evaluation will report both an estimation of the repeatability and of the factor dependant repeatability (or intra-laboratory reproducibility) of the method [3].

This example illustrates the intra- laboratory validation of a method and also the possible needs of round robin test in order to i) confront a method developped in one laboratory to other existing SEC methods (including solvent, columns, calibrants ...) ii) define a method for round robin test and standardization.

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ANALYSIS OF DISTRIBUTION OF GMA(GLYCIDYL-METHACRYLATE) IN PP GRAFTED WITH GMA USING SEC-FTIR AND MULTIVARIATE ANALYSIS

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Introduction

Maleic acid anhydride (MAH) grafted to PP is commercially available, and it is widely used as a compatibiliser in the formation of alloys of polyolefins and other polymers. The advantage of GMA compared to MAH is the possibility to compatibilise a wider range of polymers, and it has a faster reaction rate in the compatibilisation stage.

The main purpose of using SEC-FTIR in analysis of PP-g-GMA is its ability to measure the distribution of GMA on the polymer backbone. Another advantage is that the SEC fractionation also removes non reacted material like free GMA. A standard FTIR analysis of the total polymer requires a tedious washing procedure to remove monomers and non reacted materials. GMA is detected by the carbonyl group having a stretch frequency in the $1700 cm^{-1}$ region. The carbonyl stretch frequency has a large extinction coefficient and is easily detectable even at low concentrations.

Experimental

To provide a successful grafting of GMA onto PP both styrene and a peroxide, in addition to GMA, is added to the extruder during the grafting process. The samples analysed on SEC-FTIR have different combinations of GMA, peroxide and styrene at varying concentrations. Two samples are also included to study a processing parameter. The samples were analysed on 4 linear mixed bed columns (Waters 150C) connected to a LC-Transform (Lab Connections). The FTIR analyses were performed on a Perkin Elmer 1760 equipped with MCT detector.

Data analysis

Selected parts of the spectra were decomposed using principal component analysis (PCA). The score values reflect the differences in absorbance between the spectra. The C-H stretch region $(3000 - 2770 cm^{-1})$ was chosen to represent the amount of C-H in the backbone, and the region $1700 - 1800 cm^{-1}$ was decomposed to give a value of the carbonyl region. The intensity of the carbonyl peak is divided by the intensity of C-H to get a ratio of carbonyl on polymer backbone. No polymer bonds overlap in frequencies with the carbonyl peak.

Results and conclusion

Styrene was not detected in the FTIR spectra of PP-g-GMA. If styrene was grafted onto the polymer backbone, the amount is so low that it is below the noise level of the method.

Level of carbonyl.

If we want to compare the level of C=O in several samples, it is best to choose the spectra from the part of the run with highest absorbance of C-H (most material eluting from the SEC columns), which also is the region with best signal-to-noise ratio. The use of peroxide has shifted the SEC-curves towards lower Mw, and thus it is useful to plot the distribution of C=O/C-H of the samples where the C-H stretch is most intense. The results show that best grafting effect is achieved when both styrene, peroxide and GMA are added during extrusion. Increasing styrene content together with GMA gives a better grafting efficiency. With no GMA added the samples still contain some C=O

due to oxidative degradation, but the samples contain no more C=O than SEC-FTIR analysis of a standard PP homopolymer. The method ranks samples according to the content of carbonyl, and the method gives valuable information, although the system is not calibrated to give Mw and MWD. The method does not give the exact number of carbonyl pr. 1000 C. This is possible if calibration samples with well-known amount of C=O pr 1000 C exists. It is also possible to use extinction coefficients from literature to calculate the concentration of C=O.

Distribution of carbonyl.
A plot of the carbonyl and C-H distributions together gives a view of the distribution of GMA on the polymer backbone, see figure 1.

Figure 1. Distribution of C=O and C-H in PP-g-GMA.
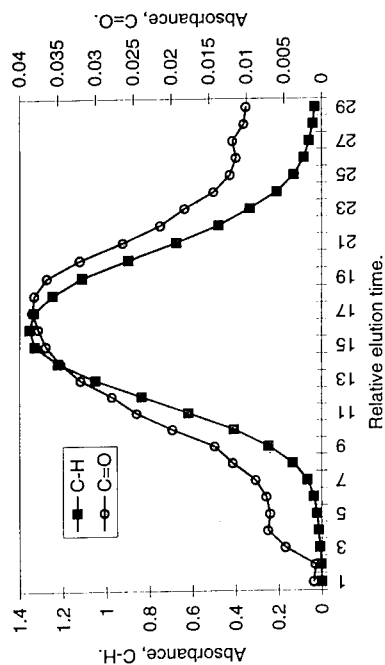
The distribution of carbonyl seems to be broader than the distribution of C-H. This is evident in all samples, including the samples with no GMA added. The explanation is oxidative degradation. The surface layer of polymer is to some extent degraded by oxygen, no matter the thickness of the polymer deposition. With low amount of polymer deposited on the disk a great percentage of polymer will be degraded, but with a thick polymer layer the surface layer only accounts for a small percentage of the polymer. The distribution of carbonyl in the studied samples does not differ from the distribution of C-H stretch, thus the samples display a random distribution of GMA on the polymer chain.

EXPERIMENTAL CORROBORATION OF THE UNIVERSAL NATURE OF ADSORPTION EFFECTS IN LIQUID CHROMATOGRAPHY OF POLYMERS

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Chromatographic distribution coefficients, K, have been obtained for polyethylene glycols, polypropylene glycols, and polystyrenes, based on the use of different adsorbents at various temperatures, mixed organic and aqueous buffer eluents of different composition. The experimental results are interpreted from the standpoint of a general theory of macromolecular chromatography, accounting for adsorption interactions. Comparison between experiment and theory has made it possible to determine correlation lengths of adsorption, H, at different pH values, temperatures, and in different mixed eluents. Since the H parameter is directly related to the mean thickness of polymer layers formed during in- pore adsorption of macromolecules, polymer layer thicknesses have been determined experimentally for various polymer-adsorbent systems.

According to the general theory of chromatograhy, the parameter $U = (K - K_{GPC})/(1 - K_{GPC})$ is an universal function of the ratio of gyration radius R to correlation length H. In fact, the entire set of experimental data fit the same U vs R/H relationship. Predicted from theory, the universal law of polymer chromatography in the presence of adsorption effects has been corroborated experimentally for most diverse systems, viz.: polymers of various chemical natures and molecular weights, adsorbents having various structures and pore sizes, aqueous and organic eluents, as well as for different methods to realize and change adsorption interactions.

STUDY OF RADICAL POLYMERIZATION AT HIGH CONVERSIONS
I. GPC MEASUREMENTS OF MOLECULAR MASS DISTRIBUTION

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It is a commonly known fact, that at high conversions the overall polymerization rate undergoes an autoacceleration leading to so called gel effect. In extreme cases it may be ended with explosion of the polymerizing system. As it is difficult to follow the elementary processes connected with radical polymerization in the range of high conversions, the nature of this phenomenon has not been completely cleared yet, though several interpretations are found in the literature. The statement about the decisive role which the increasing viscosity of the system plays in it has been confirmed by few authors, who investigated the polymerization of PMMA. This polymer is formed practically without any chain transfer and its mechanism of chain termination includes both the combination of radicals and the disproportionation mode. Selecting a solvent to which a chain transfer is excluded we tried to follow the characteristic of the products on dependance of monomer concentration and degree of conversion.

Radical polymerization of methyl-methacrylate was performed in solution of monomer in benzene at four different concentrations embracing a broad range of 10 - 90 mole percent. The reaction initiated in several dilatometers by AIBN at the same time and the same temperature of 50°C was stopped at conversions from about 5-10% up to values, where it was possible, of 60-90%. Average molecular weights and intrinsic viscosities were measured using THF as eluent in GPC system of five u-styragel columns (10E5-50nm), Waters pumping and injection system, and Viscotek dual detector (RI/DV). The results show that up to the concentration of 50 mole percent of monomer both intrinsic viscosity and molecular weights of polymer product remain constant even in case of conversions as high as 90%. At 75 mole percent of monomer a significant increase of both viscosity and average molecular weights is observed over the conversion of 25%. The values of polydispersity (U=Mw/Mn) remain practically the same. At monomer concentration of 90 mole percent the increase of viscosity is pronounced even at conversions lower then 25%, but the GPC investigation of molecular masses in this case is limited by the resolution of the columns. The application of Mark Houwink parameters quoted by the literature and those accounted from the viscosity measurements permit the statement, that the number of long chain branches in the polimer product is negligible. The ratio of rate constants of recombination to disproportionation reactions at conversion of 25% was estimated and it agrees with the data of other authors.

GPC RESULTS ON MOLECULAR CHARACTERIZATION OF DIBUTYRYLCHITIN

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Dibutyrylchitin (DBCH) is newly obtained derivative of natural chitin according to the scheme:
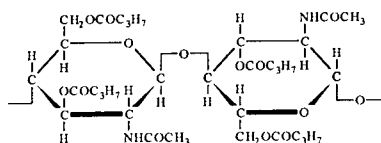
The polymer has potential technical application because of its fiber and film forming properties. DBCH is easy soluble in several organic solvent like acetone, ethanol, THF, DMF and should be able to characterize by GPC method on conventional equipment. In the preliminary approach chromatograms of unfractionated samples prepared in various experimental conditions were obtained.

GPC measurements were run using the WATERS modular equipment (pump model 510, refractive index detector model 486, Ultrastyragel columns arranged in series: linear, 10^4 Å, 10^3 Å and Baseline 810 software). THF as a solvent with adjusted flow rate 1ml/min. in ambient temperature was used. Calibration was based on polystyrene narrow molecular weight distribution standard from Tosoh Co. (molecular weight range: 3000 to 3800000). Third order polynomial curve was applied. Calculated polystyrene equivalent molecular weight averages agreed with reproducibility ±10% with those established for the same samples in Rapra Technology LTD in England. The reasonable correlation between average molecular weight and reaction conditions was observed.

It has been hoped that GPCl/viscosity approach would produce more informative data. For this purpose fractions of BCD with M_W/M_N in the range 1,2 to 1,4 were obtained by precipitation method. Their chromatograms were recorded as described above. Viscometric measurement were performed off-line in Ubbelode viscometer. Intrinsic viscosity of DBCH fractions was measured in THF and acetone at 25 C^0. The sequence of intrinsic viscosity i.e. $[\eta]_{acetone} > [\eta]_{THF}$ was observed for all sample measured. The correlation of $[\eta]$ vs. the elution volume corresponding to the maximum chromatogram height was observed. Molecular weight distribution (MWD) function of fractions can be approximated with log-normal MWD with accuracy to 3%. Data obtained were used to evaluate Mark-Houwink constants of DBCH in GPC solvent by iterative method assuming the universal calibration principle for system under investigation and linear structure of DBCH chains. The extended Mark-Houwink equation:

$$[\eta] = KM_x^a q^{apx}$$

where

q - *polydispersity degree*

x - *denotes the type of M average*

was taken into account. The validity of assumptions made has been discussed.

HPLC SCREENING METHODS FOR REVEALING THE STEREOSELECTIVITY OF REVERSIBLE BINDING INTERACTIONS BETWEEN LIGAND ENANTIOMERS AND A BIOPOLYMER

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Recently we have theoretically established three novel HPLC screening methods for studying the stereoselectivity of the reversible binding interaction between a low molecular weight ligand, its enantiomers, and a counterpart high molecular weight substance - e.g. an oligo- or polysaccharide, protein[1].

The proposed designs of the 3 different HPLC arrangements can be characterized as follows:

(i) Achiral HPLC column plus a chiroptical detector;
(ii) Chiral HPLC column plus a chiroptically insensitive detector;
(iii) Achiral and chiral HPLC columns, connected in series with a switching valve, plus a chiroptically insensitive detector.

The first arrangement (i) has been assayed for a system containing dl-tryptophan (1.0×10^{-4} mol/l) as the ligand dissolved in phosphate buffer (0.067 mol/l, pH 7.4) thus serving as the HPLC mobile phase. The stationary phase (HPLC column) was that of LiChrosorb Diol (150 x 4.6 mm). The samples assayed were proteins and their fragments.

Since the sensitivity of the chiroptical detector "CHIRALYSER" (IBZ Messtechnik) used was between 1 and 10 μg of the d-/l-tryptophan enantiomer injected onto the column, this instrumental arrangement was not sensitive enough. The introduction of a new generation of HPLC polarimeters/circular dichrographs, would however render this study design universally applicable.

The second arrangement (ii) has been elaborated by working with bovine serum albumin (BSA) as the chiral HPLC stationary phase (150 x 4.6 mm). The same phosphate-buffered dl-tryptophan solution was again used as the mobile phase. The stereoselectivity of interactions between different biopolymers and the two tryptophan enantiomers was evaluated from the areas of negative recorder deflections at the retention times of the d- and l- isomers of tryptophan[1].

The BSA column was found to be applicable in analyzing nonionic/uncharged oligosaccharides, such as α-, ß-, gamma-cyclodextrins (CD) and their derivatives. On assaying a protein or its fragments, as the counterpart substance, we observed a strong adsorption of the injected samples to the BSA chiral HPLC stationary phase, yielding severe limitations for the evaluation of the chromatograms.

Finally, the third arrangement (iii) shows the broadest applicability at present. The tandem work with achiral (LiChrosorb Diol) and a chiral (human serum albumin - HSA) columns (both 150 x 4.6 mm) was successfully utilized in measuring the enantioselectivity of the reversible binding interaction between the two tryptophan enantiomers and a number of albumins - originating from different animal species - and albumin fragments (A, B, C, D and Bp), originating from man[2].

It is really the latter experimental design (iii) which should be proposed both for present use and further development on combining as an achiral column that of LiChrosorb Diol, or another biofluid compatible sorbent, and as a chiral HPLC column that of:

a) bound BSA or HSA for acidic (negatively charged) or zwitterionic ligands;
b) bound α_1-acid glycoprotein (α_1-AGP) or ovomucoid (OVM) for basic (positively charged) ligands;
c) bound CD, or another water compatible chiral selector, on working with noncharged or weakly charged ligands.

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SEC ANALYSIS OF RAPIDLY DEGRADING POLYMERS

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Autooxidative degradation which is known for poly(methylacetylene) [1] and poly(phenylacetylene) (PPhA) [2-6] and which may be expected also for other substituted polyacetylenes represents a phenomenon which should be taken into account for any molecular-weight (MW) characterization of these polymers.

Using various SEC techniques it was found for high-molecular-weight PPhA that this polymer is stable in vacuum but undergoes a rapid degradation (of the random character) in air, which is in solution ca hundred times as fast as in the solid state [3,6]. For example, the $\bar{M}_w$ value of freshly dissolved PPhA sample (in THF) was found to drop from 710 000 (injection into SEC device 15 minutes after mixing PPhA with THF) to 370 000 after the storage of the solution in air for two hours only (injection 135 minutes after the mixing).

High rate of PPhA degradation implies that attention should be paid to the storage of this polymer and, particularly, to the preparation and treatment of solutions used for MW determination. In addition, it was found by SEC experiments with various flow rates that the degradation of dissolved PPhA did continue to proceed inside the SEC columns during the separation process. This implies a question how this phenomenon affects the results obtained by means of individual SEC evaluation techniques (PS calibration, universal calibration, absolute detection). It is worth mentioning that this questions is important not only for PPhA but has a general meaning for MW characterization of any polymer degrading inside the SEC columns.

An attempt has been made to solve the problem of the degradation of a polymer during the SEC analysis theoretically. Two methods were applied, (i) computer simulation of the random degradation of macromolecules in a column, (ii) numerical solution of a set of differential equations describing a degradation in the course of SEC separation including the axial dispersion. On the base of these simulations the MW distribution and indexes of non-uniformity in MW can be calculated for each slice of SEC chromatogram. Results confirm the increase in MW non-uniformity of slices of SEC chromatograms due to the degradation during the SEC separation.

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POSSIBILITIES OF POLYMER CHARACTERIZATION BY GPC WITH ON LINE FTIR - OR NMR-DIRECTION

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Sometimes the characterization of oligomers or polymers in low molecular weight range especially the determination of molecular weight averages by size exclusion chromatography (SEC) is due to different solvatation behaviour of this substance. Such effects, which are caused by different chemical structures, monomer sequences in the macromolecule or in increasing influence of end groups, do not allow to use normal molecular weight calibration relationships.

In contrast to the well known off-line application of spectroscopic methods like FTIR and NMR and separation techniques, a direct on-line detection using FTIR and NMR, was carried out during the SEC run to proof the different influences on the chromatographic separation process.
As expected, FTIR and NMR as detecting methods give information about the the chemical structures of the elueted species. In addition, it was possible to determine molecular weights for different chromatographic fractions. Molecular weights up to 7000 g/mol could be calculated from the NMR spectra of different fractions.
The preferences and limits of these on-line couplings of spectroscopic methods with the liquid chromatography will be discussed using an oligomeric polyethersulphone as example.

DILUTE SOLUTION PROPERTIES OF POLYURETHANE IONOMERS

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Introduction

Dilute solution properties of ionomers are strongly influenced by the polarity of solvents, by the type and content of ionic groups, by temperature, by polymer molar mass and concentration. Among these, solvent polarity has a very important role: less polar solvents solvate ion-pairs and the molecular associates are formed, while more polar solvents promote ionization and the expansion of macromolecules due to the repulsion forces of ionic groups occurs. This phenomenon is known as a polyelectrolyte effect and can be suppressed by the addition of salts like LiBr, LiCl, LiNO$_3$ etc[1-4].

In this work the SEC analysis of model polyurethanes (PU), synthesized from poly(tetramethylene oxide) (PTMO), hexamethylene diisocyanate (HDI) and 2,2-bis(hydroxymethyl)propionic acid (DMPHA) and/or 2,2-dimethyl-1,3-propanediol (NPG) as chain extenders is presented. Elution behavior of the PU containing -COOH groups was studied in different solvents, tetrahydrofuran (THF), N,N´-dimethylformamide (DMF) and in LiBr+DMF, and was compared to the neutralized PU (ionomer) and to PU without DMPHA. To elucidate the SEC elution process, viscosity behavior of the polyurethanes was also investigated.

Experimental

Synthesis: PU were synthesized via prepolymer procedure as 40% solutions in DMF at 80°C. The reaction products were precipitated from water and dried in a vacuum oven. The molar ratio of PTMO: HDI: chain extenders was 1:3:2. The concentration of DMPHA in the chain extender mixture is shown by the designation D X, X being the mole percentage of DMPHA. Ionomers were prepared by neutralization with hydroxides.

Measurements

SEC measurements were performed at room temperature on a Perkin Elmer liquid chromatograph with a LC-30 differential refractometer. Eluents THF, DMF, DMF + LiBr at a flow rate of 1.0 mL/min and PLgel 5μm column Mixed D were used. The solution concentration was in all cases 1% (w/v) and the injection volume 20 μL. The calibration curve for the eluent THF was made by means of polystyrene standards. For DMF and DMF+LiBr only elution volumes, V_{el}, are reported.

The reduced viscosity was measured by Ubbelohde viscometer at 25±0.05°C.

Results and Discussion

SEC results of the model PU in THF, DMF and DMF+LiBr are given in Tables 1 and 2. It can be seen that the changes in hydrodynamic volume are in correlation with the degree of carboxylation and of ionization. In THF, the molar mass averages decrease (V_{el} increase) with the increased concentration of

DMPHA and are the lowest for the PU ionomer. It is known that in less polar THF, at low concentration of PU ionomers, the solution behavior is governed by intramolecular interactions and ionomer coils become more compact[1,3]. This is also reflected in solution viscosity behavior: intrinsic viscosity $[\eta]$ of the ionomer is smaller than that of its precursor without DMPHA and of PU with DMPHA.

Table 1. Molar mass averages, polydispersity index and intrinsic viscosity of model PU in THF

PU	M_w	M_n	M_w/M_n	V_{el}, mL	$[\eta]$, mL g^{-1}
D 0	57,000	29,000	2,0	6.42	58.18
D 50	32,000	14,000	2,2	6.87	36.93
D 100	22,000	10,000	2,2	7.01	36.32
D 50 Na	19,000	10,000	1,9	7.15	18.88

Table 2. Elution volumes of model PU in DMF and DMF+LiBr

PU	DMF	0.011M LiBr	0.05M LiBr	0.1M LiBr
D 0	6.82	6.81	6.84	6.86
D 50	5.85	7.76	7.37	7.34
D100	5.34	8.51	7.80	7.74
D 50 Na	*	7.87	7.45	7.49

    * multimodal peak distribution

In DMF, PU with DMPHA elute earlier than expected from the steric exclusion mechanism. Anomalous SEC behavior is ascribed to intramolecular chain expansion and intermolecular association. Multimodal peak distribution of carboxylated PU and ionomer are ascribed to different charge distribution or number in the polymer chains[2].

With the addition of LiBr to DMF, the chromatograms of carboxylated PU and ionomer have more symmetrical shape and much higher elution volumes than in pure DMF. V_{el} stabilizes in 0.05M and in 0.1M LiBr, where it does not differ significantly, which means that the ionic charges on polymer chains are effectively screened in 0.05M LiBr already. This has been also confirmed by viscosity measurements. PU ionomer exhibits characteristic polyelectrolyte behavior in DMF. The relationship between the reduced viscosity $[\eta]_{red}$ and concentration of PU with DMPHA and ionomer is linear in 0.05M and 0.1M LiBr.

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SEC-LALLS STUDY OF THE PPHA DEGRADATION. INFLUENCE OF THE TYPE OF THE CATALYST USED FOR PPHA SYNTHESIS ON POLYMER STABILITY

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INTRODUCTION

Stability is the key factor limiting a practical use of polyacetylenes in electronic devices. However, surprisingly little attention has been paid to the degradation of these polymers. Besides poly(acetylene) and poly(methylacetylene) [1], only poly(phenylacetylene) (PPhA) has been studied in this respect more in detail [2,3,4]. PPhA was found to be stable in vacuum, but to undergo a rapid auto-oxidative degradation in both solid and dissolved state (THF, chloro-benzene). The kinetics of degradation was studied in details for PPhA samples synthesized with $WOCl_4/2Ph_4Sn$ catalyst system and was found to obey the theoretical relations valid for the random degradation [5]. The fact that this degradation takes place without any induction period was ascribed to the easy initiation of this process by direct interaction of oxygen with both mobile and immobile neutral defects (unpaired electrons) of conjugated chains [1,3].

In this contribution, the results of SEC study of PPhA degradation (in THF solution exposed to the atmosphere) are reported and an influence of the catalyst used for PPhA synthesis on the degradation behaviour of polymers is discussed. Our preceding studies show that the degradation of high molecular weight PPhA is so rapid that a decrease in molecular weight is meaningful even 'or times corresponding to the duration of SEC analyses [3,6]. Since we have ecently found that the degradation continues to proceed inside the columns during the SEC separation process [7], interpretation of SEC data obtained for PPhA by means of calibration techniques may be complicated. Due to this reason, the application of LALLS detector coupled with SEC was found to be very advantageous and this technique was used in the present study.

EXPERIMENTAL

Synthesis: PPhA samples were prepared by polymerization of PhA with various metathesis catalyst systems: $Mo(CO)_6$, (I), $W(CO)_6$, (II), WCl_6/Ph_4Sn, (III), $WOCl_4$, (IV), $WOCl_4/2Ph_4Sn$, (V), and $WOCl_3$ (O-2,6-di-t-Bu-C_6H_3), (VI). Dry argon atmosphere and molar ratio PhA to catalyst of 100 were applied for syntheses with I - III; standard vacuum break seals technique and molar ratio PhA to catalyst of 2000 for syntheses with III - VI.

Equipment: SEC data were obtained with a Waters 150C chromatograph fitted with a LALLS photometer Chromatix CMX-100 and a standard Waters differential refractometer. A series of PL-gel columns (10^6, 10^5, 10^4, 10^3 and

$5x10^2$) and THF as a mobile phase with a flow rate of 1 mL/min at 25°C were used. PPhA sample (stored as solid in vacuo) was allowed to dissolve in THF for 30-40 min before the first SEC injection. Afterwards, the solutions were kept at 25°C and successive SEC injections were taken at a given time.

RESULTS AND DISCUSSION

The random character of degradation was confirmed for PPhA samples prepared by polymerization of PhA with I, III, IV and V. This fact is demonstrated by the linearity of $1/\bar{X}$ vs. degradation time (t) dependencies ($\bar{X}$ is either the number average or the weight average degree of polymerization). The slope of these dependencies is either equal (for $1/\bar{X}_n$ vs. t) or proportional (for $1/\bar{X}_w$ vs. t) to the rate constant of a bond rupture in a main chain of PPhA, ν, [3] and thus it can be considered as a measure of the rate of polymer degradation. Values of ν_n as ascertained from $1/\bar{X}_n$ vs. t are given in Table 1. For PPhA samples prepared with II and VI a deflection from linearity of $1/\bar{X}_n$ vs. t was observed (in Tab. 1, the initial values of ν_n estimated for $t \rightarrow 0$ are given). This fact indicates not a purely random character of degradation of these samples. The above differences in degradation behaviour will be discussed in connection with PPhA microstructure.

Table 1. Values of $\bar{M}_n$ and $\bar{M}_w$ of freshly dissolved PPhA (the first SEC analysis) and values of ν_n for PPhA prepared with various metathesis catalyst systems

Catalyst system	$10^{-3} \cdot \bar{M}_n$	$10^{-3} \cdot \bar{M}_w$	$10^6 \cdot \nu_n$ (min^{-1})
Mo(CO)$_6$, (I)	28	52	0.8
W(CO)$_6$, (II)	36	67	(9)
WCl$_6$/Ph$_4$Sn, (III)	35	67	4.7
WCl$_6$/Ph$_4$Sn, (III)	250	400	4.3
WOCl$_4$, (IV)	88	170	6.2
WOCl$_4$/2Ph$_4$Sn, (V)	500	850	2.0
WOCl$_3$(O-2,6-di-t-Bu-C$_6$H$_3$), (VI)	130	230	(10)

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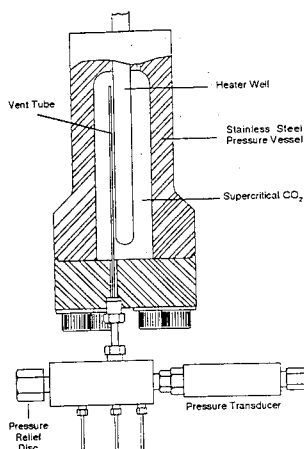
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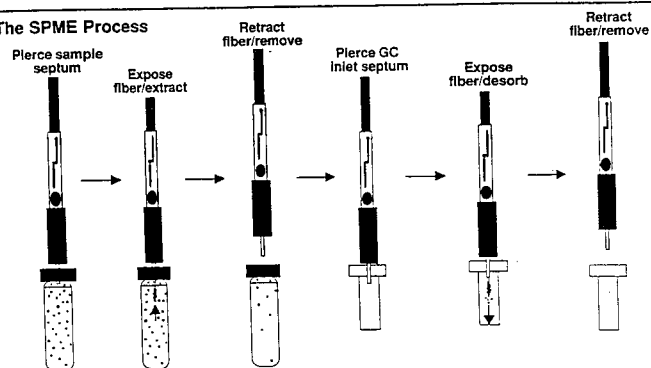
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